NATURE

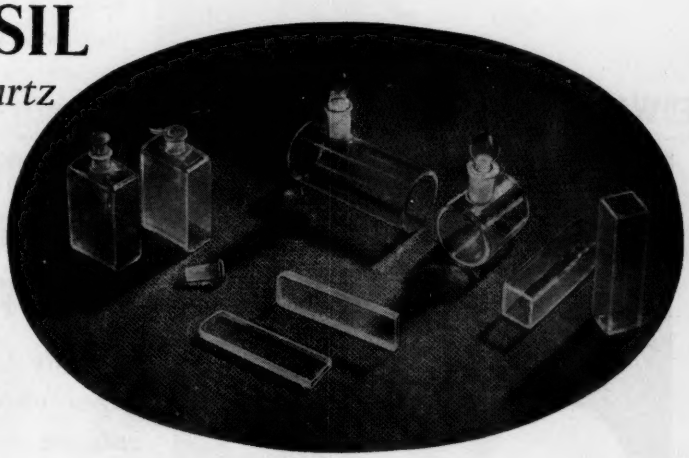
A Weekly Journal of Science

UNIVERSITY OF MICHIGAN
APR 28 1959
PERIODICAL READING ROOM

Vol. 183, No. 4667 SATURDAY, APRIL 11, 1959 2s. 6d.

VITREOSIL

pure fused quartz



VITREOSIL
SPECTROPHOTOMETER CELLS FOR ULTRA-VIOLET ABSORPTION RESEARCH

Vitreosil Spectrophotometer Cells are made from specially selected transparent fused quartz, which has a very high transmittance in the ultra-violet range. All our cells are thoroughly fused and there are no cemented joints. The standard sizes, suitable for all spectrophotometers, can be supplied singly or in matched groups.

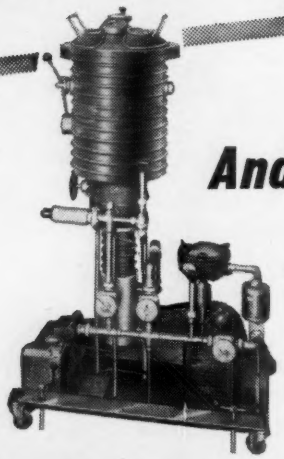
Note—Vitreosil Hydrogen Lamps provide a continuous source of ultra-violet radiation

TSL

THE THERMAL SYNDICATE LTD.
P.O. Box No. 6 WALLSEND, NORTHUMBERLAND *Telephone: Wallsend* 6-3242
LONDON: 9 BERKELEY STREET, W.1 *Telephone: Hyde Park* 1711/2

LABORATORY VACUUM FURNACE

(½ lb. capacity — steel)



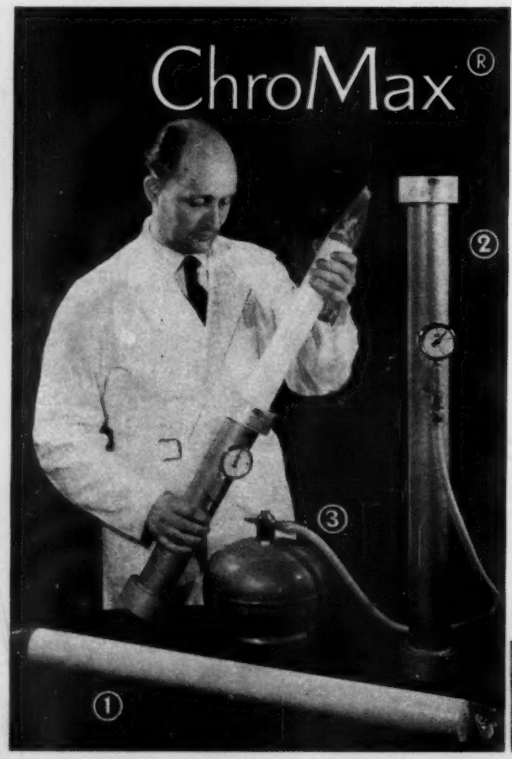
MELTING
CASTING
SINTERING
ANNEALING

And Now — VACUUM BRAZING

The well-known "SPEEDIVAC" vacuum furnace, used throughout the world for small-scale production and investigation into vacuum melting and heat treatment of metals, is now available with an additional STATIC RADIATION HEATER ASSEMBLY. This assembly not only enables a thorough investigation of vacuum brazing problems but also provides a production unit for a wide variety of small components.

This unit, which can be supplied as an accessory to existing ½ lb. (0.23 kg.) furnaces, has a uniformly heated 'hot-zone' 2 in. diameter and 4 in. high. Similar facilities can also be supplied with Edwards large size vacuum furnaces.

EDWARDS HIGH VACUUM LTD · MANOR ROYAL · CRAWLEY · SUSSEX · ENGLAND · Crawley 1500



Preparative Column Chromatography the easy way

with the ChroMax *pressurized paper chromatography column system*

Ready-made paper roll insert (1) loads easily into pressure mantle (2) which is inflatable by means of pressure ballast flask (3).

Insert and mantle are available in a range of sizes to meet the need for differing separation capacities. ChroMax columns are capable of separating in gram quantities all such mixtures as are separable on a microscale on single sheets of paper – and with the same definition!

U.K. Distributor:
A. Gallenkamp & Co. Ltd., London, E.C.2.

LKB **LKB-PRODUKTER**
Fabriksaktiebolag
P.O. Box 12220, Stockholm 12, Sweden

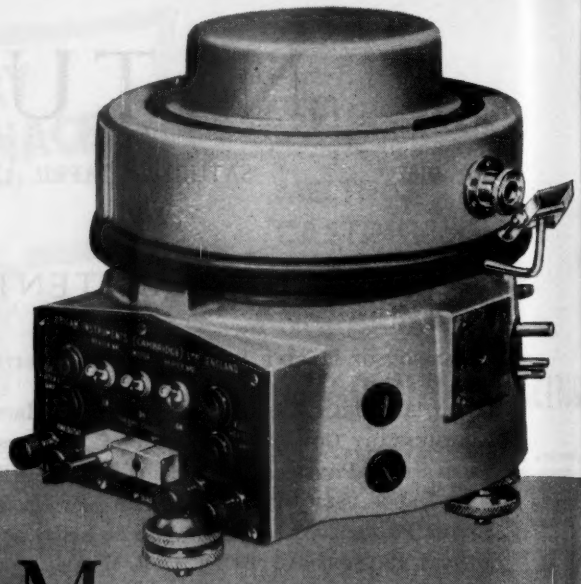
NATURE

No. 4667 SATURDAY, APRIL 11, 1959 Vol. 183

CONTENTS

	Page
Expansion of Marine Research in the United States	1005
Early American Naturalists. By Dr. L. Harrison Matthews, F.R.S.	1006
Rudimentary Government. By Prof. M. Fortes	1006
Solid State Physics. By Prof. G. F. J. Garlick	1007
New Drugs used by Psychiatrists. By Dr. Marthe Vogt, F.R.S.	1007
Soviet Psychology. By Dr. Peter McKellar	1008
Muscle Physiology. By Dr. D. R. Wilkie	1008
Inorganic Micronutrients in Physiology and Agriculture. By Dr. E. J. Hewitt	1009
The Nuffield–Witwatersrand University Expeditions to Kalahari Bushmen, 1958–59. By Prof. P. V. Tobias	1011
The Medical Research Reactor at the Brookhaven Medical Research Center. By Dr. E. E. Stickley	1013
Obituaries:	
Prof. Arturo Duperier. By Prof. P. M. S. Blackett, F.R.S.	1015
Prof. Pierre Fauvel. By Dr. Norman Tebble	1016
Dr. Herbert Hunter. By Dr. G. D. H. Bell	1016
Dr. Alexander M. Cockburn. By Prof. F. H. Stewart	1017
Dr. S. Bekku	1017
News and Views	1018
The Carnegie Institution of Washington	1025
The National Research Development Corporation	1026
Figural After-effects. By Prof. H. J. Eysenck	1027
Iron Ore in the Soviet Union. By Prof. S. I. Tomkeieff	1028
Employment of Scientists and Technologists in the United States	1028
Chemotherapy of Parasitic Diseases. By Dr. June Mahon	1029
A Presumptive Human *XXY/XX* Mosaic. By Dr. C. E. Ford, Dr. P. E. Polani, J. H. Briggs and Dr. P. M. F. Bishop	1030
Transphosphorylating Enzymes and Muscle Relaxation. By J. Molnar and Dr. L. Lorand	1032
Mechanism of Action of Diphosphopyridine Nucleotidases. By Prof. Spyridon G. A. Alivisatos	1034
Molecular Adaptation in Hæmoglobins: Nature of the Bohr Effect. By Austen Riggs	1037

(Continued overleaf)

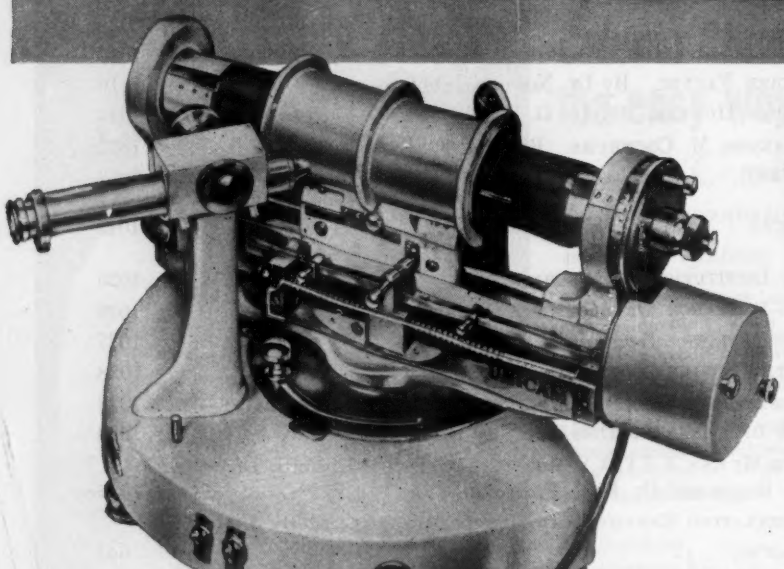


S.70
HIGH TEMPERATURE POWDER CAMERA

—provides an economical, accurate and, through its control unit, easily operated instrument for high temperature powder work up to 1000°C. The camera used is a standard 19cm. Debye-Scherrer type.

UNICAM

CRYSTALLOGRAPHIC INSTRUMENTS



S.35
SINGLE CRYSTAL WEISSENBERG GONIOMETER

—is a versatile instrument precision-made to provide accurate information of crystal cell sizes and the indices of reflecting planes. It can be used for all normal techniques requiring film movement synchronously with crystal oscillation.

Please write for illustrated brochure and price list.

UNICAM INSTRUMENTS LIMITED · ARBURY WORKS · CAMBRIDGE

U171

LETTERS TO THE EDITORS

Page

CHEMISTRY

New Oxidation States of Technetium.—J. E. Fergusson and Prof. R. S. Nyholm, F.R.S. . 1039

Cyclic Alternating-Current Voltammetry with a Platinum Electrode.—André L. Juliard . . 1040

Polymorphic Conversion of Mercuric Iodide on Alumina.—Prof. Harry Zeitlin and Harry Goya 1041

Solution of Iodine in Phosphorus(III) *Iso*cyanate.—Dr. Ervin Colton and Lewis St. Cyr . . 1042

Interaction of 1,3,5-Trinitrobenzene with Ethoxide Ion.—Dr. R. Foster 1042

Relation between the Polarizability of Ions and other Significant Properties.—Prof. K. Krishnamurti 1043

PHYSICS

Nuclear Magnetic Resonance in Rolled Copper Sheet.—E. A. Faulkner 1043

Movement of Auroral Echoes and the Magnetic Disturbance Current System.—R. S. Unwin . 1044

Atmospheric Tides and Earth Satellite Observations.—D. G. Parkyn; G. V. Groves . . 1045

Time Relationship of Metre-Wave and 3-cm. Wave Bursts.—M. R. Kundu . . . 1047

Atmospheric Dust and Fluorescence.—Dr. K. Pribram 1048

The Three-dimensional or Solid-Image Microscope.—W. D. Jackson 1048

GEOPHYSICS and GEOCHEMISTRY

Remanent Magnetism of the Cretaceous Basalts of Madagascar.—Dr. A. Roche and L. Cattala . 1049

Biological Origin of Manganese-rich Deposits of the Sea Floor.—John W. Graham and Susan C. Cooper 1050

BIOCHEMISTRY

Amino-Acetone: its Isolation and Role in Metabolism.—Dr. W. H. Elliott 1051

Identification of Barbiturates.—Dr. A. S. Curry . 1052

Isomerization of Poly-*cis*-carotenes by Chlorophyll *in vivo* and *in vitro*.—Dr. H. Claes and T. O. M. Nakayama 1053

Green Fluorescence of Guanidinium Compounds with Ninhydrin.—Dr. Rex B. Conn, jun., and Dr. Richard B. Davis 1053

A Simple Method for the Concentration of Fluids containing Protein.—Dr. J. Kohn . . . 1055

Unfermentable Sugars in Barley Mash fermented by the *Amylo Process*.—*Hideo Ono* and Michio Dazai 1055

Selective Inhibition of Formation of Deoxyribonucleic Acid in *Escherichia coli* by Mitomycin *C*.—S. Shiba, A. Terawaki, T. Taguchi and Prof. J. Kawamata 1056

Page

ANIMAL PHYSIOLOGY

Biosynthesis of Ovalbumin.—Prof. D. V. Siva Sankar and Henry W. Theis 1057

Molecular Orientation at the Surface of *Amoebae* during Pinocytosis.—Ronald C. Rustad . . 1058

Re-utilization of Lymphocyte Remnants by Reticular Cells.—Dr. M. Hill 1059

A Survival Curve for Mammalian Cells irradiated *in vivo*.—Dr. H. B. Hewitt and C. W. Wilson . 1060

Relative Rates of Liver Storage of Pure α-, β-, γ- and δ-Tocopherols in the Growing Chick.—T. W. Griffiths 1061

Normal Components of Guinea Pig Serum which may cause Antigens to show an Apparent Anticomplementary Effect.—M. M. H. Sewell . 1062

Audio-direction Finding in the Porpoise (*Phocaena phocaena*).—W. H. Dudok van Heel . . 1063

PLANT PHYSIOLOGY

Effect of Light on Maturing Broad Bean Seeds.—Dr. G. R. Lane and R. D. Butler . . . 1063

Differential Reaction of Saprophytic and Parasitic Soil-inhabiting Fungi to Indoleacetic Acid.—E. W. B. Ward and Prof. A. W. Henry . . 1064

Formation of Purple Pigment in *Spirogyra pratensis* Cultures.—Dr. Ann Allen and Dr. R. E. Alston 1064

BIOLOGY

Dissociated Nystagmus in the Rabbit.—Dr. J. Lachmann and Prof. F. Bergmann . . 1065

Strain Differentiation in a Root-infecting Fungus.—Dr. Ida Levisohn 1065

Forest Seedlings and Mycorrhiza Development on Fly Ash.—A. Sobotka and J. Materna . 1066

Analysis of Mast Cells by means of Polarization Microscopy.—Prof. Leslie Horváth . . 1067

PATHOLOGY

Oxygen Exposure and Brain Damage.—Lars Gyllensten 1068

Role of the Thymus in Murine Leukæmia.—J. F. A. P. Miller 1069

Plaque Production with Yellow Fever and Related Arthropod-borne Viruses.—J. S. Porterfield 1069

BACTERIOLOGY

Bacteriophage Enrichment in a Liquid Two-Phase System with Subsequent Treatment with 'Freon' 113.—Dr. G. Frick and Dr. P. Å. Albertsson 1070

Chloramphenicol and the Survival of Airborne *Bacteria*.—*S. J. Webb* 1072

Photomicrograph supplied by Dr. M. J. Olney and reproduced by courtesy of the University of Cambridge, Department of Metallurgy.

The metallurgist's plate

During the course of experiments involving the heat treatment of hypereutectoid steels, observations through a microscope showed that a grey-black film was formed on the surface of the steels. This happened when the steels were heated into the austenitic region and cooled in high vacua.

The film was identified as graphite, related crystallographically to the austenite grain structure which the steels possessed at 1,000°C.

Further experiments proved that the graphite formation was caused by a reaction which can take place in inert atmospheres on the surface of steels with high carbon content—no trace of graphite was formed in the interior of the steels.

In these experiments, 'Kodak' 0.250 plates were used to provide the necessary information.

The 0.250 plate is the acknowledged standard for photomicrographic investigations in the field of metallurgy. It provides particularly good definition in that part of the spectrum where it is most needed. It has ample speed and unobtrusive grain. It is the metallurgist's plate . . .

0·250 Kodak Rapid Orthochromatic Metallographic Plate

Kodak Limited, Industrial Sales Division, Kodak House, Kingsway, London, W.C.2

AUTOMATIC TITRATIONS

ARE easily carried out by the E.I.L. Automatic Titrimeter, Model 24. This instrument is fully automatic, switching itself off on reaching the pre-set end point.

Either the volumetric or the coulometric method may be used, and separate titration units for each type are available. Duplicated connections are provided on the Control unit.

The endpoint setting dial is calibrated in pH and in millivolts, and the equipment can be used for any type of chemical reaction producing a determinate change of e.m.f. The accuracy of titration is 0.05 c.c. in 50 c.c.

E·I·L

the pH people

ELECTRONIC INSTRUMENTS LIMITED RICHMOND SURREY

E.9a

EXPANSION OF MARINE RESEARCH IN THE UNITED STATES

THE National Academy of Sciences – National Research Council has issued a statement making known the conclusions of a Committee which was set up at the request of a number of Government agencies to review the progress and needs of marine science in the United States. The full report is promised in the near future. The main conclusions are encouraging because they show that many of the problems that have long been too large and perplexing to attract close attention are becoming more approachable and promising; they are also disturbing because they show that progress, though commendable, does not match growing needs for detailed information and understanding of the oceans.

The statement mentions the need for increased food supplies. The main factor leading to high fertility in the oceans is active mixing and upwelling from the deep layers, where nutrients are regenerated, to the illuminated surface layer where the plants grow. Upwelling is not confined to coastal regions, or to oceanic islands and shoals, and it can occur wherever there are sharp differences and changes in the surface and sub-surface currents. Such areas, far from land, are already forming the basis of ocean fisheries, and the factory ships that will fish them will need good understanding of the fundamental physics and biology as well as the breeding, feeding and migratory habits of the fish to ensure economical working with regular catches.

The need for more understanding of fluctuations and trends in climate is also emphasized. The heat capacity of the oceans is very much larger than that of the atmosphere, and the oceans are largely responsible for the thermostatic control that makes so much of the Earth habitable. They gather the Sun's heat and distribute it as moisture-laden air in a pattern determined by complex interaction between sea and air, currents and winds. The slow but powerful changes in the oceans have important effects on agriculture, fisheries, ice conditions and sea-level. All the great hurricanes and typhoons start over the sea.

Modern studies of waves, tides, currents and movements of sediments have much to offer to ship designers, coastal and harbour engineers, and to those concerned with special aspects of navigation, salvage and oil drilling. The National Academy Committee, however, gives special emphasis to the needs of military defence. With every fresh advance in surface, amphibious and undersea warfare, the side with the fullest knowledge and most detailed understanding of the oceans is likely to gain some advantage in devising new techniques, discarding out-of-date ones and judging how to proceed. The report mentions the submarine menace and maintains that "from the point of view of military operations there is no comparison between the urgencies of the problems of the oceans and those of outer space". Concern that the new fascination of space will divert American scientists and the Government from the vast unexplored regions and problems of the oceans is implied throughout the statement.

The study of the sea floor has made tremendous strides during the past five years. With the help of new tuning-fork controlled echo-sounders the floor of the deep ocean can be charted in nearly as much detail as a river estuary, and the half-inch to a mile maps, photographs, cores and dredges reveal fascinating new information about active movements of water and sediment, eroded channels, possible mineral resources and the habits of bottom-living animals. The time has come when geology can be actively extended to the rocks and sediments at the bottom of the oceans.

The main recommendations of the Committee are that basic research in marine science should be doubled within the next ten years, and that the research effort should be supplemented by ocean-wide surveys. The resources of the present surveying effort, mainly that of the U.S. Coast and Geodetic Survey, would be doubled for the purpose. The whole programme would be strengthened by expanded application of the science to military defence, marine resources and the problems raised by the use of nuclear energy.

The first step towards the Committee's objective is the provision of new ships with the character of floating laboratories, and others for survey. The need for new devices such as submarines, mid-ocean platforms and unmanned buoys is emphasized, and new instruments and telemetering equipment are needed. It is estimated that each ship needs sixty shore-based scientists and technicians, and the difficulty of attracting men with the essential skill and enthusiasm is clearly recognized as the greatest difficulty.

Oceanography is no longer a descriptive subject; some of its theoretical and practical work is as advanced, as difficult and as likely to find useful applications in other sciences, as that done in any research laboratory. The subject has begun to attract attention in universities, especially in the United States, and the Committee feels that its difficulties may be surmounted by more deliberate measures to encourage interchange with universities. There can be no subject of wider and more rapidly growing interest or of more potential world value, and yet there is a shortage of qualified postgraduate research workers because the specialized facilities that might be used to continue university training are not used to the full, and the universities have few of their own. This problem will become more acute as the work in progress outside the universities requires increasing numbers of workers with more and more specialized qualifications; to solve it the Committee advocates affiliation of oceanographic

research institutions with university faculties, and the development of new centres at universities.

The Committee proposes a ten-year plan costing 651 million dollars over and above the present level of expenditure. The plan is described as 'minimal' to meet long-range requirements, and it is stated that action on a scale appreciably less "will jeopardize the position of oceanography in the United States relative to the position of the science in other major nations, thereby accentuating serious military and political dangers, and placing the nation at a disadvantage in the future use of the resources of the sea".

The report has no official Government approval, but opinion in the United States expects it to guide the course of national support for marine science. Oceanographic research has already been earmarked by the President's Science Advisory Committee as one of the major scientific fields needing more emphasis. Further afield it is also of interest. From its very nature, oceanography is dependent on, and contributes to, most of the other sciences; moreover, its pursuit involves international collaboration on an extensive scale. Any increased support in the United States for oceanography will contribute to the development of the science and set an example which other countries might well follow.

EARLY AMERICAN NATURALISTS

John and William Bartram's America

Selections from the Writing of the Philadelphia Naturalists. Edited with an Introduction by Helen Gere Cruickshank. Pp. xxii+418. (New York: The Devin-Adair Company, 1957.) 5 dollars.

The Travels of William Bartram

Edited with commentary and an annotated index by Francis Harper. Naturalist's edition. Pp. lxi+727+42 illustrations and facsimiles. (New Haven, Conn.: Yale University Press; London: Oxford University Press, 1958.) 68*s*. net.

THE Quakers John Bartram and his son William were two of America's leading naturalists throughout the eighteenth century—their combined lives cover a span of nearly one hundred and twenty-five years, from 1699 until 1823. Although they were primarily botanists, and John established the first botanic garden in America near Philadelphia about 1730, they were widely interested in all aspects of natural history. In their early days there were few books that could help them in classifying the numerous undescribed species that they found, but when a merchant friend in Philadelphia took some of John's collections and observations to Peter Collinson of London, who immediately appreciated their value, a correspondence was started that lasted nearly fifty years. As a result of John's friendship with Collinson he entered into correspondence with nearly all the naturalists of Europe, including Linnæus and Sir Hans Sloane, and was elected into the fellowship of the Royal Society, and appointed "Botanist to His Majesty for the Floridas".

Both the Bartrams travelled extensively in pursuit of their studies, venturing far beyond the scattered settlements of the outposts into Indian territory where they endured many discomforts and hardships and not a few dangers. In 1743, John accompanied an expedition to make a treaty of peace with the Iroquois and travelled north to Oswego on the shores of Lake Ontario, but his major explorations were made twenty years later in Florida, a region which made a deep impression upon both him and his son. Afterwards, William also spent five years travelling through the Carolinas, Georgia, and Florida, gathering specimens and recording his observations. John was brought up to farming and had very little schooling; although he kept diaries of his travels he did not publish them and they were incorporated into the accounts printed by other members of his expeditions. William Bartram, however, had a fluent pen, and in 1791 he published at Philadelphia his "Travels through North and South Carolina, Georgia, East and West Florida", which was at once reprinted in London and Dublin, and translated into several foreign languages. This book, containing most interesting topographical, anthropological and biological observations, reveals him as a delightful character, full of curiosity, sympathy, and an appreciation of wild Nature rare in those days when the age of elegance regarded everything remote from the centres of civilization as a dreadful wilderness full of savage and horrid prospects.

The first of the volumes under review is an anthology of writings by, and about, the Bartrams, and is an excellent introduction for the general reader to the works of these pioneers. "Although William was one of the foremost of the earliest generation of American writers on natural history and travel, his work has never been known and appreciated in America the way it was abroad." There is no longer any reason for the prophet to be without honour in his own country. The second book is very much more ambitious. It is a reprint of the first edition of William's travels with a long and interesting introduction by Francis Harper, and a valuable commentary identifying the places visited, and the plants and animals seen, based upon extensive journeys made over Bartram's trails and upon much literary research. There is also a copious annotated index, an interesting section of illustrations and a number of maps. William Bartram's narrative is a fascinating account of America nearly two hundred years ago, and Harper's faithful editing enhances its lustre.

L. Harrison Matthews

RUDIMENTARY GOVERNMENT

Tribes without Rulers

Studies in African Segmentary Systems. Edited by John Middleton and [the late] David Tait. Pp. xi+234. (London: Routledge and Kegan Paul, Ltd., 1958.) 28*s*. net.

SEGMENTARY political systems constitute one of two main types of indigenous political organization found in Africa. Unlike the centralized type, they have no forms of general tribal government vested in chiefs or courts of law or any other overriding authority. They are made up of a collection of mutually independent segments, most commonly based on descent, which maintain law and order among themselves by various forms of self-help, including often the feud, and by making use of kinship, local and ritual bonds. Tribes of this type were, in the 'thirties, frequently recalcitrant to the

efforts of colonial governments to devise for them administrative patterns in line with the aims of indirect rule. A handicap here was the paucity of ethnographic information on African political institutions and the consequent inadequacy of theoretical understanding of how they worked.

A beginning was made in the systematic study of the subject when, in 1940, Prof. Evans-Pritchard and I brought out a collection of essays describing a representative series of African political systems ("African Political Systems", edited by M. Fortes and E. E. Evans-Pritchard, 1940). The distinction between the centralized type and the segmentary type was there suggested. Post-war field research has so extended the range and quality of our ethnographic knowledge of Africa that this typology is now seen to be too broad. More thorough analysis of each type is now possible, and the present book makes a welcome beginning with this task. All the contributors belong to the post-war generation of social anthropologists. The six societies they deal with in the book were barely known before the Second World War. Certainly their indigenous political institutions could not then have been analysed with the technical proficiency and theoretical understanding shown by the editors and their fellow contributors.

The peoples described in the present book live in West Africa, the Sudan and East Africa. They vary from sedentary cultivators occupying clearly defined villages, like the Bwamba of Uganda, admirably described by Dr. Winter, to the transhumant cattle-keeping Dinka of the Sudan, whose complex social structure is skilfully elucidated by Dr. Lienhardt, and the widely dispersed, near-anarchic, Konkomba farmers and fighters of Ghana, described with great insight by the late Dr. David Tait, one of the editors. They differ widely in language, custom and belief. What they have in common is a segmentary political structure based on some form of kinship and descent group.

In addition to the six essays, there is a valuable introduction by the editors. They demonstrate that the segmentary type really includes a range of varieties. They propose a division into three groups, an important distinction being the manner in which the genealogical and local segments are constituted and combined into what they call "jural communities". The analysis of the economic, military, juridical and religious sanctions correlated with this classification is particularly instructive. The book is a significant addition to the growing body of field research on non-western political institutions. It is also, as Prof. Evans-Pritchard remarks in his preface, a fitting memorial to Dr. Tait, who died in a motor accident before it was published. M. FORTES

SOLID STATE PHYSICS

Solid State Physics

By Prof. Adrianus J. Dekker. Pp. xiv + 540. (London: Macmillan and Co., Ltd., 1958.) 45*s*. net.

IN recent years various text-books on solid state physics have been published, not without some influence on each other. At first sight, particularly with respect to selection of topics, Prof. Dekker's book bears a likeness to that of Kittel. However, more careful inspection reveals a different appreciation of the various aspects of the subject. There is evidence of the skilled teacher of modern physics with an awareness of the large range of mathematical ability among his student readers. The first nine chapters require little knowledge of the electronic characteristics of solids, beginning with a brief introduction to crystallography and moving all too rapidly through specific heats of solids, lattice defects, dislocation theory, dielectrics, ionic conduction and ferroelectricity. However, the presentation in each case is skilful enough to promote curiosity and wider reading in more specific texts. A further feature is the stressing of fundamentally important matters, for example the emphasis on the Debye theory of specific heats, though with proper indication of the more complex and, practically, almost unwieldly problem of lattice vibration spectra. To mention another chapter, that on ferroelectrics is lucidly written especially in the reference to the thermodynamic theory.

It is natural that the electronic properties of solids find a large place in this book. Non-polar crystals are emphasized, together with their applications in transistors. Complete chapters are devoted to luminescence and secondary electron emission, respectively, while the last part of the book deals with modern magnetism, inclusive of resonance phenomena and their importance in solid state research. If one looks for faults, and these are few, I feel that omission of dielectric breakdown might be remedied in the future and there might also be afforded a little more space for treatment of types of binding in solids.

Each chapter carries a number of exercises or problems; there are an adequate subject index and some useful appendixes. One more favourable comment must be added, about the surprisingly modest price of the volume compared with similar publications and with its equivalent price in the United States. G. F. J. GARLICK

NEW DRUGS USED BY PSYCHIATRISTS

The New Chemotherapy in Mental Illness

The History, Pharmacology and Clinical Experiences with Rauwolfia, Phenothiazine, Azacyclonol, Mephenesin, Hydroxyzine and Benactyzine Preparations. Edited by Dr. Hirsch L. Gordon. Pp. xviii + 762. (New York: Philosophical Library, 1958.) 12 dollars.

THIS book is a collection of 119 articles by 167 authors, the majority of whom are American psychiatrists, relating their personal experiences with one or several of the new drugs used in mental illness. The individual writers address themselves to the practising physician and give their impressions, views, recommendations and warnings based either on the material they may have had at their disposal or on their personal philosophical outlook. This leads to statements so contradictory that they become rather disconcerting. Thus one author states on p. 99 that combined therapy with chlorpromazine and reserpine produces a prohibitively high incidence of serious untoward effects, whereas another worker assures us on p. 397 that this combination lacks toxicity and side effects, and he recommends it (p. 400) in neuropsychiatric treatment because of its "advantages over the use of either agent when employed alone". A third group of authors (p. 423) takes the intermediate view that the combination of the two drugs is probably not superior to the use of either drug

alone, but that the toxic effects of larger doses are enhanced. That views on therapeutic successes are notoriously apt to be based on faith rather than on fact explains such contradictory opinions; yet methods of clinical trial are available nowadays which overcome these difficulties and yield reliable insight. A few carefully conducted trials, such as that described on p. 13 by H. A. Bowes who was trying to assess the value of azacyclonol ('Frenquel'), are more valuable than any number of uncontrolled clinical impressions. There are many valuable and well-founded observations in the book, but each article has to be scrutinized for the way in which the conclusions were reached before the reader can accept them as valid. The first seventh of the book contains articles of a somewhat more general nature than the later papers which relate clinical experiences only, but even in this section papers based on clinical impressions only are found side-by-side with others of a more objective nature.

If this book is to guide the practitioner through the labyrinth of neuropharmacology, care must be taken over nomenclature. The table on p. xvii is seriously misleading. Thus it suggests that 'Largactil', 'Megaphen' and 'Thorazine' are different compounds because it lists them alphabetically with other phenothiazines without revealing that they are identical. Even more confusing is the placing of 'Meratran' under the heading 'azacyclonol' and of meprobamate under the heading 'mephenesin', as this suggests that, for example, 'mephenesin' could be used as an alternative and more general name for meprobamate.

The main drugs covered by the book are shown in the title; more than 75 per cent of the material deals with reserpine and chlorpromazine.

Marthe Vogt

SOVIET PSYCHOLOGY

Psychology in the Soviet Union

Edited with an Introduction by Brian Simon. Translated by J. and M. Ellis, J. McLeish, H. Milne, N. Parsons and others. (International Library of Sociology and Social Reconstruction.) Pp. viii+305. (London: Routledge and Kegan Paul, Ltd., 1957.) 32*s*. net.

THIS book derives from the work of the Russian physiologist Pavlov, to whom the Western world accords the respect due to a great scientist. It comprises twenty-two papers contributed by contemporary Soviet psychologists and selected as representative by Prof. A. A. Smirnov and N. A. Menchinskaya of Moscow. Their emphasis is upon human rather than upon animal behaviour, and the studies are frequently in terms of the development of the individual.

The field covered by the nineteen Soviet contributors deals with the principal areas of interest to their Western counterparts. Prof. B. G. Ananiev (Leningrad) deals with problems of perception; Prof. L. V. Zankov (Moscow) with memory, and Prof. E. A. Milerian (Kiev) with attention. The paper on psychopathology by Prof. A. R. Luria (Moscow) suggests close research association of Soviet psychologists with neurophysiologists and psychiatrists. Several papers deal with thinking, apparently a subject of considerable interest to the Russian investigators.

It is on the subject of learning that British and American psychologists have derived most from Pavlov's work: the learning process is now given a central place in the subject-matter of modern experimental psychology. The papers in this field deal with the subject somewhat differently and principally in an educational context, rather than in terms of the more usual technique of the Western psychologist, namely, in the context of animal experiments. Thus, for example, Prof. N. A. Menchinskaya (Moscow) sets out to show how the principles governing learning can be applied to improvement of methods of instruction, and reports experiments on the use of visual techniques in training people to think abstractly in mathematics and elsewhere.

Psychology, presented from the point of view of dialectical materialism, exhibits certain characteristics which merit contrast with Western psychology. The Russian work emerges as heavily experimental; the method of psychometric measurement that is also prominent in British psychology is noticeably absent. Again, some of the contributors, notably Prof. B. M. Teplov (Moscow), reject the idea current in Western psychological theory that principles and laws are not "immutable" and "objective" parts of Nature but simply tools for making predictions about natural phenomena. In their theory the Russian contributors exhibit a distinct preference for theoretical concepts of a physiological kind. Contemporary Western theorists, by contrast, are sharply divided as to whether basic units of analysis should be physiological or of some other kind (for example, logical or mathematical).

A paper by Prof. A. A. Smirnov (Moscow) assesses achievements and omissions of Soviet work in the period 1953–55. Here and elsewhere, one notes the wide range of complex psychological processes to which Pavlovian concepts and techniques have been applied: these applications will certainly interest British and American investigators. Despite the explicit rejection of the method of introspection by Prof. Teplov, Prof. Smirnov makes a plea for more study of—among other things—the complex problem of differences of mental imagery. He also makes reference to the need for more study of the history of world psychology, and for more application of the results of completed researches.

A concluding paper gives a Russian assessment of the Fourteenth (Montreal) International Congress of Psychology and indicates that interaction between Western and Soviet psychology is in progress. This book should stimulate more such interaction. The editor, contributors and translators merit our thanks for this valuable and interesting book.

Peter McKellar

MUSCLE PHYSIOLOGY

Die Muskeltätigkeit

Versuch einer Biophysik des Quergestreiften Muskels. Von Prof. Eugen Ernst. Pp. 355. (Budapest: Verlag der Ungarischen Akademie der Wissenschaften, Akadémiai Kiadó, 1958.) n.p.

IN the introduction to his book, Prof. Ernst describes the difficulty that he had in deciding to spend a year away from his laboratory in order to write; but there is no doubt that he has done a most admirable and helpful thing, in setting down for us his results and thoughts after forty years work in muscle physiology. It happens all too seldom that a

veteran in any field will make the effort to write down, let alone tidy up, the developments in his science throughout his lifetime.

He writes, understandably enough, with an intense sense of personal involvement. He evidently feels that his own contributions have been under-estimated and quotes with some bitterness "The publicity each theory has received has depended almost as much on the strength of personality of its protagonist as on its intrinsic merit".

Prof. Ernst's disputatious style, and his wide reading, combine to give a vivid historical picture of the way in which muscle physiology has developed. In addition, the experimental work for which he is best known—on the volume changes in muscle and on the osmotic activity of water in muscle—is presented in some detail; while numerous other aspects of muscle physiology are reviewed or discussed. Sometimes he does not give enough experimental detail to permit a firm answer to an important question, for example (p. 46), where he reports that isolated myofibrils are excitable electrically; and although some of his criticisms seem to me to be just—for example (p. 266), where he points to the confusion that surrounds the concept of muscular efficiency—he sometimes goes too far, or makes mistakes himself. Thus, in claiming priority over A. V. Hill for the discovery that when an active muscle is stretched it can absorb energy (pp. 11, 321), he seems unaware that his mechanical measurements alone, without heat measurement, cannot possibly solve this problem.

The book is well produced, but there is a cumbersome system of cross-referencing (to chapters and sections instead of to pages) that may have made it easier to write, but which certainly makes it more difficult to read. An infuriating example occurs on the last page (330), where it is implied that Hodgkin and Horowicz have failed to make proper reference to Prof. Ernst's work. To investigate this, a laborious search through the book is necessary, which ultimately arrives back on the self-same page from which it started, where it is found that the vital piece of evidence, by Laszlo, is still unpublished.

Nevertheless, it is Prof. Ernst's sincere interest in comprehending his subject that permeates and dominates his book. The fuel for his polemics seems to be the irritation that he feels because, after so many years work, by so many different people, the field still abounds in uncertainties, contradictions and misinterpretations. D. R. WILKIE

INORGANIC MICRONUTRIENTS IN PHYSIOLOGY AND AGRICULTURE

Trace Elements

Proceedings of the Conference held at the Ohio Agricultural Experiment Station, Wooster, Ohio, October 14–16, 1957. Edited by C. A. Lamb, O. G. Bentley and J. M. Beattie. Pp. xii+410. (New York: Academic Press, Inc.; London: Academic Books, Ltd., 1958.) 12 dollars.

THE volume under review comprises 23 chapters concerned with quite different aspects of trace-element nutrition, and a summary by Dr. C. A. Elvehjem given at a three-day symposium, which although rather unequal in treatment probably present in some respects more informed accounts than could be achieved by a single author.

Provocative contributions by Arnon dealing with the requirements and roles of trace elements with special reference to vanadium, molybdenum and manganese in different plant organisms, and by Hutner and his collaborators describing a novel approach to methods for revealing nutrient requirements by increasing metabolic stress with some emphasis on cobalt, present what may be termed the philosophy of trace-element investigations.

The chapters on selenium in soils, plants and animals by Moxon, on cobalt requirements by Davis, and on cobalt in B_{12} synthesis by Johnson and Bentley are both timely and informative. Iron is discussed by Granick, zinc by Hoch and Vallee, and copper mainly in relation to uricase by Mahler. Monoiodotyrosine metabolism is described by Serif and Kirkwood, but the use of bare references in place of certain details on methods and previous work are more akin to a paper of a series than a symposium contribution.

The specific role of boron in plant nutrition is still unknown. Skok, however, gives an excellently concise and detailed account of present knowledge regarding boron. Shorter papers by Sayre, and by McLean and Volk, deal with boron in corn and alfalfa.

Nason contributes a comprehensive statement regarding the roles of molybdenum and vanadium in plants and animals. The following paper by Keeler and Varner on molybdenum- and tungsten-protein fractions in *Azotobacter* approaches the problem of whether molybdenum fulfils a single or multiple role in *Azotobacter*.

The chapter by Pirson on manganese in photosynthesis reactions of higher plants and green algæ is a welcome summary regarding the apparent role of manganese in this complex process. Shorter papers discuss manganese problems in soils and in soybeans (Hoff and Medersky), manganese in grape production (Beattie), and manganese toxicity in apple (Winter).

Two chapters by Brown, Eyster and Tanner deal comprehensively with the mineral nutrition of *Chlorella pyrenoidosa* and especially with the differential effects of manganese in heterotrophic and autotrophic growth, respiration and photosynthesis. These papers, with those of Pearson, Arnon, Nason, Hutner and collaborators, Johnson and Bentley, and Keeler and Varner, comprise a fairly comprehensive account of micronutrient metabolism in micro-organisms.

Some minor omissions are of references to both sodium and chlorine in the index in spite of allusions in the text and the indexing of rubidium and fluorine. Reference to work of Brown and Possingham (1957) on iron and cell division is omitted from the text, pp. 378–9 and the index, though it is included in the bibliography, and the main paragraphs on hæmoglobin, pp. 368 and 370, are not indexed. The printing is excellent, and there are probably in all more than 1,000 different references conveniently distributed in relation to each chapter.

This book is recommended as a necessary part of any library used by students and teachers of plant physiology and of mineral nutrition in any field. Research workers will find therein much information on a wide range of trace-element problems and some stimulating or provocative points of view which may encourage further investigations. E. J. HEWITT

Observation and Interpretation

A Symposium of Philosophers and Physicists. Edited by S. Körner in collaboration with M. H. L. Pryce. (Proceedings of the Ninth Symposium of the Colston Research Society held in the University of Bristol, April 1st–April 4th, 1957.) Pp. xiv+218. (London: Butterworths Scientific Publications; New York: Academic Press Inc., 1957.) 40*s*.; 8 dollars.

THE conference recorded in this volume brought together a remarkably distinguished international gathering of philosophers and physicists. There were seven sessions, at each of which two short papers were presented, and the discussion which followed was tape-recorded.

The main topic is indeterminism in quantum physics, with the problems arising from it. Several of the papers, including those of Profs. Braithwaite, Ayer and Popper, deal with the notion of probability, and discuss whether the frequency view is adequate for use within physics, and how to express the degree to which a belief is supported by evidence. The central argument develops with Profs. Bohm and Rosenfeld as antagonists, and leads to the question whether the laws of quantum mechanics would be the same whether there were human observers or not. Bohm outlines his view that the indeterminacy of quantum physics is not necessarily ultimate but may be explicable by deterministic behaviour at a deeper level, somewhat as random Brownian motions are explained by deterministic motions of molecules. Among the most interesting of the other papers are that of Prof. Körner on regulative principles in physics and that of Mr. Kneale on the question whether we can 'see' atoms.

Most of the papers could be read by anyone interested in the philosophy of science or in quantum physics. The discussions give the reader the excitement of a debate by experts on live and important issues. The volume is a successful experiment in the reporting of an important symposium.

E. F. Caldin

Science in New Zealand

Edited by F. R. Callaghan. Pp. 272+27 plates. (Wellington, N.Z.: A. H. and A. W. Reed; London: Bailey Bros. and Swinfen, Ltd., 1957.) 22*s*. 6*d*.

AS with the British Association in Great Britain, it has become a tradition for the Australian and New Zealand Association for the Advancement of Science to issue a hand-book on the occasion of its periodical meetings. The holding of the thirty-second meeting in Dunedin in January 1957 was marked by the issue of the present volume. It makes no attempt to be comprehensive in its scope, and its articles aim at dealing with only a selected group of scientific interests. They are written in a style readily understandable by all readers and provide a general background knowledge of the scientific and cultural features of New Zealand.

A distinct local colour and flavour are regarded as an essential feature of an A.N.Z.A.A.S. hand-book and the present volume is descriptive, historical and scientific throughout its chapters.

Among articles of outstanding interest is one by Gilbert Archey on the relationships between Maoris and Polynesians, native and introduced birds by E. C. Turbott, geothermal power by G. W. Grindley, aerial top-dressing by E. A. Gibson, trans-Tasman relationships in natural history by C. A. Fleming, and the growth of hydro-electric schemes by F. R. Callaghan. There is also a valuable introduction showing the contributions made to the development of science in New Zealand by Sir Joseph Banks and Sir Joseph Hooker.

The book is well illustrated, not the least important photograph showing a group of Maori intellectuals considering the new edition of the standard dictionary of their language.

Micro-Organisms in the Soil

By Prof. Alan Burges. (Biological Sciences.) Pp. 188. (London: Hutchinson and Co. (Publishers), Ltd., 1958.) 10*s*. 6*d*. net.

FEW books have been written on soil microbiology since the Second World War, and therefore the present volume, although much biased towards soil mycology, will be most welcome to soil scientists. Workers in general in biological and related sciences should also find something to interest them.

Prof. A. Burges has taken upon himself a heavy task, and he is to be commended for approaching it from the ecological point of view. The opening chapter, which gives a brief account of the soil and its structure, is followed by a chapter on the various groups of organisms found in the soil. No diagrams or photographs are provided and consequently some of the descriptions of the more complicated organisms (for example, nematodes) and the systematics (for example, of the algae) will be fully appreciated only by those trained in zoology and botany. The succeeding chapters deal with such topics as: methods of examining the soil population; form, distribution and ecology of the organisms in the soil; the role of micro-organisms in the biochemical transformations taking place in soil; the influence of man on soil micro-organisms; and a consideration of soil as a dynamic equilibrium system. It is in these chapters that the true worth of the book will be found.

This volume will be very useful to students who have to learn something about soil microbiology and who are looking for an up-to-date account in a comprehensive form. The references at the end will be found of particular value in this connexion.

D. M. Webley

A Treatise on Photo-Elasticity

By Prof. E. G. Coker and Prof. L. N. G. Filon. Second edition revised by H. T. Jessop. Pp. xxxvi+720. (Cambridge: At the University Press, 1957.) 70*s*. net.

THE Cambridge University Press has performed a very real service to all those interested in the theory and history of photoelasticity by producing a second edition of Coker and Filon's classic work on this subject; this was first published in 1931 and has been out of print for many years.

The book has been reprinted by the offset-litho process; it differs from the first edition, apart from a very few minor corrections and alterations, in only two particulars. The colour photographs contained in the original edition have been omitted, presumably to keep down the cost of the new edition, and the reviser, Col. H. T. Jessop, of University College, London, has added an excellent short introduction giving an outline of developments in the field of photoelasticity since 1931 and a short bibliography covering this period.

This new edition is a book which technological and scientific libraries as well as individual workers in photoelasticity will wish to place on their shelves.

E. K. Frankl

THE NUFFIELD–WITWATERSRAND UNIVERSITY EXPEDITIONS TO KALAHARI BUSHMEN, 1958–59

By Prof. P. V. TOBIAS
Convener, Kalahari Research Committee, University of the Witwatersrand

DURING the winter of 1958 and the summer of 1959, two expeditions from Johannesburg carried out the most comprehensive series of field-studies ever made on the biology of the Kalahari Bushmen. The full collated reports will ultimately form the subject of a special monograph, but a provisional report on the background, organization and achievements of the investigation is presented here.

The Kalahari in Bechuanaland comprises a large area of sand veld, with slight rainfall, scanty vegetation, practically no agriculture, a sparse and often nomadic population and a virtual dependence of man on domestic and wild animals. It includes, too, the swamp-lands and broad rivers of the Okavango inland 'delta', which Prof. J. H. Wellington regards as potentially one of the chief food-producing areas of southern Africa.

The peoples of Bechuanaland fall into the following ethnic categories: (1) Bantu-speaking Negroids; (2) Hottentots; (3) Bushmen; (4) Europeans (Caucasoids); (5) Bastards—a variety of blends of the other four groups.

Of these five categories, the serious decline in numbers of the Bushmen over the past three hundred years, and especially in the last century, has made the study of this group the most urgent. Although recent estimates have placed the number of surviving Bushmen at as high a figure as 50,000 (Tobias, 1956), it has been deemed necessary for a number of reasons to give the highest priority to the Bushmen, in a systematic investigation of the inhabitants of the Kalahari. At present they are being subjected to rapid change in their conditions of life; to progressive blurring of their tribal boundaries in some areas, with consequent cultural and genetic detribalization; and to the impingement of other races, languages and cultures upon their genetic structure, click language and subsistence economy based on hunting and food-gathering.

For more than thirty years the Department of Anatomy of the University of the Witwatersrand has been studying the indigenous population of South Africa in general and the Bushmen in particular, especially the divergences in the skeletal structure of the Bush and Negroid peoples. These skeletal studies have been supplemented by a number of field-surveys over the past twenty years, as a result of which much information has been collected about living Bushmen (see, for example, Dart, 1937). Meanwhile, other studies had been devoted to linguistic, psychological, cultural and medical aspects of the Bushmen, as well as, in more recent years, their applied physiology. For many years, these field-surveys had been carried on in an unsystematic and desultory fashion.

Recent years have seen a considerable revival of interest in the physical anthropology of the Bushman and Hottentot peoples. As a consequence, no fewer than thirty-four papers bearing on this topic were published during the period 1952–55, compared with seven works in this category in the years 1948–51. Studies on the living have included physical and serological accounts of Nama Hottentots, Strandlopers, Koranas, Sandawe, Lake Chrissie Bushmen, Northern Bushmen, River Bushmen, Central Bushmen, and Bushman hybrids.

To co-ordinate and systematize the investigations, as well as to accelerate the work, in the face of rapid changes in the circumstances of the Bushmen, a Kalahari Research Committee has come into being at the University of the Witwatersrand. Its members are drawn from those departments of the University which have been concerned with the Kalahari researches, as well as from two collaborating institutions, the Applied Physiology Laboratory of the Transvaal and Orange Free State Chamber of Mines (director, Dr. C. Wyndham) and the National Institute for Personnel Research (director, Dr. S. Biesheuvel), a specialized institute of the South African Council for Scientific and Industrial Research.

The Kalahari Research Committee has initiated a systematic investigation of the inhabitants of the Kalahari Desert, among whom first place is being given to the Bushmen.

With the aid of a generous grant from the Nuffield Foundation, as well as substantial assistance from the University of the Witwatersrand and a grant from the S.A. Council for Scientific and Industrial Research, the Committee was able to mount the first two expeditions in August–September 1958 and January 1959.

The area selected was Ghanzi, where researches had previously been made on Bushmen (Tobias, 1955–56; Clement, Plotkin and Fosdick, 1956). Around the District Commissioner's camp at Ghanzi, and along a line of European- and Bastard-owned farms running westwards from Ghanzi to the border with South-West Africa, two major linguistic sub-groups of the Bushmen meet: to the north and west are the much-studied Northern Bushmen of Dorothy Bleek, whereas to the south and east are the little-known Central Bushmen. The population studied on the 1958 and 1959 expeditions comprised predominantly Central Bushmen, with a smaller proportion of Northern Bushmen. All the branches of study represented on the expeditions were in the broad realm of human biology.

Physical anthropological studies. These were made by Mrs. H. Eriksen, of the Witwatersrand Department of Anatomy, and by Dr. J. S. Weiner, reader in physical anthropology in the University of Oxford. Very few measurements on Central Bushmen have hitherto been published, so the 150 anthropometric and anthroposcopic observations recorded on each of 86 adult males and 54 adult females constitute a valuable addition to knowledge. 81 of these individuals belong to the !x3η (Magon) tribe, which has never previously been studied by physical anthropologists. 20 were of the Naron tribe, of which a small series had previously been published by Tobias (1955–56);

while 21 were Auen (Kaukau) of the Northern Bushmen. The remainder comprised mainly inter-tribal 'hybrids' and Bushman–Negroid hybrids.

For the first time on Bushmen, skinfold measurements were taken with a Harpenden skinfold caliper. The objective assessment of steatopygia and steatomeria was successfully attempted by measurement of skin pinches over the buttocks and thighs. The use of female investigators greatly facilitated this aspect of the work, since Bushwomen are always loth to uncover their buttocks, which are a much-prized feature in their sexual economy. Detailed measurements and observations could likewise be taken on the enlarged labia minora (macronympha or the 'Hottentot apron'). Dermatoglyphic records were taken on palms and finger-tips and will provide a useful basis for comparison with similar observations collected recently on Bantu-speaking Africans from Nyasaland, Tanganyika and the Zambezi Valley in Northern Rhodesia (unpublished results).

The study of skin colour by the old von Luschan tablet has long proved rather inadequate, and an excellent opportunity was provided for a more objective assessment by the use of Dr. Weiner's portable reflectance spectrophotometer introduced in 1951. He collected observations for the skin colour on the inner aspect of the arm and on the forearm, from 142 Bushman subjects. These readings will make it possible to specify quantitatively the range of skin colour and to compare this with that of other racial groups on whom the instrument has been used (including West Africans, South African Bantu-speaking Negroids and Australian Aborigines).

Blood-groups, hitherto not assessed on Central Bushmen, were investigated on 114 Ghanzi Bushmen. For the blood-grouping, Dr. A. Zoutendyk, of the South African Institute for Medical Research, has used almost the whole range of anti-sera of anthropological value, including the recently discovered 'negroid' Js and V anti-sera (supplied by Dr. E. Giblett, of Seattle) and the 'mongoloid' Diego (supplied by Dr. T. Layrisse, of Venezuela). The results, which have been published by Weiner and Zoutendyk in *Nature* of March 21, p. 843, confirm the generally negroid pattern of Bushman blood-groups, including the occurrence of V and Js. Diego antigen is totally lacking, while the previously established points of departure of Bushman blood from that of Negroids are confirmed, namely, high frequency of A and low frequency of B and the absence of Rh-(ceddee).

Somatotype photographs, hair samples and hand-grip dynamometer readings were also collected by Dr. Weiner, while Mrs. Eriksen made plaster-face-masks of Bushmen to add to the collection of more than six hundred African life-masks built up by Prof. R. A. Dart and his assistants in the Anatomy Department during the past thirty years.

Oral and dental investigation. This was carried out by Dr. J. F. van Reenen, senior lecturer in the School of Dentistry of the University of the Witwatersrand. A clinical and morphological study was made of 278 Bushman dentitions. Nearly half the Bushmen examined had an edge-to-edge bite, generally with attrition. A high incidence of gingivitis and hypoplasia of the enamel was recorded, but the incidence of caries was low. In addition, a bacteriological investigation was made on samples of saliva from 41 Bushmen. First results indicated the presence of hæmolytic streptococci and of coagulase-positive staphylococci in a large proportion of subjects. Few were positive for yeasts and a fair number for lactobacilli.

Applied physiology. During the winter months of 1958, a team from the Applied Physiology Laboratory, University of the Witwatersrand, led by Mrs. J. S. Ward, investigated the physiological responses of Bushmen to cold. It was found that air temperatures ranged from 85° to 93° F. by day down to 29° F. at night. The difference between maximum and minimum temperatures on sunny days and clear nights might be as much as 50–60 deg. F. Relative humidity was low, and no condensation was recorded on any night. The theory has often been canvassed—not without challenge—that the Bushman is peculiarly adapted physically to life in the desert. Apart from the evidence adduced elsewhere (Tobias, 1957) that he is a late arrival on the desert scene, Mrs. Ward's team confirmed earlier observations by Wyndham and Morrison (*Nature*, 178, 869; 1956) that the Bushman's adaptation to Kalahari conditions is largely cultural, rather than anatomical or physiological. By the intelligent use of fire, shelter and individual bodily covering, the Bushmen create comfortable temperature conditions during the cold winter nights. Physiological indications are that Bushmen, like Europeans under these air conditions, suffer a fall in skin temperature and increase their metabolism by shivering.

In January 1958, a second team from the Applied Physiology Laboratory, under Dr. N. B. Strydom, studied heat responses of Bushman males to humid heat in a specially devised climatic tent. Pulse-rate, rectal temperature and loss of weight were recorded hourly during a 4-hr. stop-test in a saturated environment of 90° F.

The variable electro-cardiographic response of Africans on cooling of the precordium led Dr. B. Kaminer, senior lecturer in physiology, University of the Witwatersrand, to study with a portable cardiograph the records of 60 male Bushmen and to make precordial cooling experiments on 14 Bushmen. Arterial blood pressure readings on 102 Bushmen revealed no tendency for pressure to increase with age. In addition, water 'load' tests were made on 10 Bushmen and clinical examinations on 78 Bushmen.

Psychological performance testing. This was carried out by Dr. H. Renning, of the National Institute for Personnel Research, Johannesburg. His first problem, to determine whether the Bushmen are amenable to simple performance testing, could clearly be answered in the affirmative, and 86 males and 15 females were tested. The tests used were: (1) sand drawing; (2) pattern completion (coloured version); (3) object-relations test (Zilian); (4) Ishihara colour-blindness; (5) Müller–Lyer illusion (method of limits); (6) perceptual illusions (Herskovits *et al.*); (7) recognition of shape. Of these, only the Müller–Lyer illusion presented difficulties to the subject. All other tests, none of which required any verbal responses, appeared to give reliable results. Some tests requiring the handling of paper (for example, the 7-squares test) could not be used in the open, because of prevailing strong winds; and, in general, test sessions could not be extended beyond 40–50 min. without imposing an undue strain on the subject.

Although the evaluation of results will not be completed for some time, a few points can be made immediately:

(i) The intellectual capacity of the Bushmen tested varied over a wide range.

(ii) Personality and temperamental differences were also great and readily observable in responses to test situations. Emotional tension was marked in many subjects during the first 10–15 min. of the test session.

(iii) The ability to abstract is apparently little developed in the Bushmen. For example, whereas a simple alternation, in the object-relations test, of black/white/black/white, or square/round/square/round, was in most cases correctly continued when two pairs were presented, the series of black square/white square/black round/white round was found too difficult even when three complete cycles were laid out by the experimenter.

Dr. A. J. Clement, who led the expedition into the field and established its bases, collected further demographic data on the numbers and distribution of eight western Kalahari tribes. These data largely confirmed those collected earlier by Tobias (*1956*).

Nearly thirty years ago, Schapera (1930) wrote: "There seems little doubt that the Bushmen are steadily dying out as a race. What relentless persecution at the hands of other peoples has not achieved is being slowly accomplished by disease and racial intermixture. Racially pure Bushmen are already in a minority, and their ultimate absorption by their neighbours is probably inevitable".

Since then, though the factor of disease may have declined somewhat, intermarriage of the Bushmen with other peoples has continued apace under more settled conditions, so that both tribal and racial boundaries are rapidly becoming blurred. There is a distinct danger that the extinction of the Bushmen by hybridization is foreseeable and that, thereby, anthropologists will be robbed of the chance of gaining a most important insight into human development and adaptation. It is hoped that such a tragic default will be averted by the concerted survey launched by the Kalahari Research Committee.

THE MEDICAL RESEARCH REACTOR AT THE BROOKHAVEN MEDICAL RESEARCH CENTER

By Dr. E. E. STICKLEY

Medical Department, Brookhaven National Laboratory, Upton, Long Island, New York

AFTER nearly five years of planning and more than two years in construction, the new Medical Research Center at Brookhaven is nearing full utilization as its unique Medical Research Reactor—the first in the world—has reached critical reactivity. This event occurred on March 15 at 9.44 p.m. New York time. The critical mass was established as 2·249 kgm. of uranium-235. Operation in the full power-range was achieved on April 3.

Since the design of the reactor was fully studied during the planning and testing stage, it is expected that the machine will come quickly into experimental use, bringing the new establishment nearly to a completely functioning state. The Medical Research Center buildings were dedicated on December 16 last, and the Research Hospital was transported from the old quarters as a unit and set up completely in its new space on January 6, while moving of the laboratories was completed in mid-February.

It is significant that this reactor is the first where every salient aspect of the engineering and design of the device was considered in relation to its use in the study of man and the diseases of man. Its conception and planning were an outgrowth of the pioneering investigations using the unequalled possibilities afforded by the large general-purpose reactor at Brookhaven, where the first experiments on neutron-capture therapy and medical applications of radioactivities of extremely short half-life were carried out. Eight years of experience with this reactor, gained through the extraordinary research flexibility of that device and the unstinted aid of its scientific, engineering and operating personnel, have prepared the way for further penetration into this unexplored region of medical-nuclear research with this newest tool.

Since the Medical Research Reactor was designed specifically for use in the research programme of the medical scientists on the Brookhaven staff, the initial decisions established the quality and quantity of neutrons required to advance the technology of the earlier medical experiments. The major requirements were an increase in the slow-neutron flux, better control over the energy of the neutrons, and improved clinical convenience. From a statement of the neutron requirements, a reactor core and reflector were specified, and around them appropriate special shielding was planned to permit the utilization of the greatest possible external neutron flux. Two shielded rooms have been built, one on either side of the reactor, separated from it only by a single wall. Vertically moving shutters of 20 tons weight have been installed to control the administration of the radiation. A smaller cell has been incorporated inside the shielding for studies of the whole-body effects of nuclear reactor radiations. Other experimental arrangements include tubes into the reactor structure for production of short-lived radioactive materials, as for use in therapeutic and diagnostic studies or activation analysis; the tubes are also adaptable for collimated neutron beams, which are of value in determining the penetration and energy changes of neutrons passing through tissue-equivalent materials.

The operational criteria were best met by a modified tank-type reactor in which the neutron moderation is supplied partly by graphite in sealed cans and partly by the natural water circulated for cooling. The core is further surrounded by a reflector of air-cooled graphite approximately 1 m. thick. Initially there are 17 fuel elements, made in the Bulk Shielding Reactor design, each fabricated from 18 thin laminated plates spaced to allow passage of cooling water. A total loading of 2·377 kgm. of highly enriched uranium-235 is used. Three safety rods, each worth 2 per cent of excess reactivity, are held by magnetic clutches;

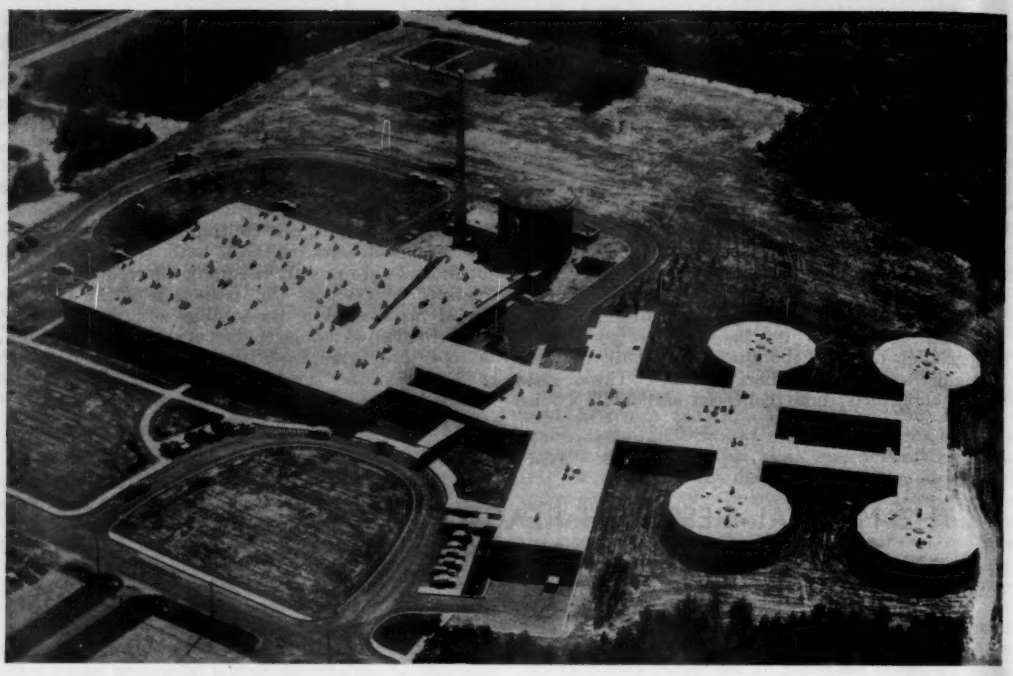


Fig. 1. Aerial view of the Medical Research Center. The reactor building appears above the centre of the picture. Research Hospital at the right. Industrial Medicine Clinic wing, lower centre. Medical Research Laboratories are at the left

they are driven into the core by gravity. Each consists of a stainless steel sheath with cadmium lining and boron carbide filling. A single control rod of similar pattern in solid steel, worth only 0·5 per cent in reactivity, is used for routine operation. At the nominal full power of 1 MW., the Medical Research Reactor is expected to have a core flux of 10^{13} and should deliver 7×10^{10} neutrons/cm.^{2}/sec. into the treatment room; this is twenty times the best flux previously available. Provision has been made for development of combinations of moderators, reflectors, filters and converter plates to select and modify the variety of radiations available in its several exposure sites.

Of these many uses and features of the Medical Research Reactor, three of outstanding immediate interest are: (1) the therapeutic application of high concentrations of slow neutrons delivered through the reactor shielding into a treatment room for the production of instantaneous radiation reactions, especially of the energetic heavy-particle variety; (2) the production of radioactive materials of very short half-life, to investigate their therapeutic and diagnostic possibilities; and (3) the development of more powerful analytical or diagnostic tests by the methods of neutron-activation analysis. In addition, other important applications lie in the use of the full range of reactor-produced radiations for studies of their comparative effectiveness in a wide variety of living systems and other materials of biological interest.

The Reactor is directly connected with the laboratories and the hospital through an air-lock corridor. To facilitate its use in the varied types of investigation for which it is expressly suited, a fully equipped operating room is adjacent, and treatment rooms for the application of high-level radioactive procedures are immediately at hand. The arrangements have been planned to eliminate any inconvenience in preparation or treatment of patients in carrying out the experimental procedures.

The Research Hospital provides a total of 48 beds in four circular pavilions, so arranged for the best control and convenience in observation and care; all the necessary additional service functions of a self-sustaining hospital are included. An Industrial Medicine Clinic occupies an adjoining wing, providing out-patient care and serving the occupational health needs of the Brookhaven National Laboratory as a working community. The Hospital and Clinic for handling patients are balanced by five related research divisions within the Department under the basic science headings of Physiology, Biochemistry, Microbiology, Experimental Pathology and Medical Physics. The nominal size of the scientific staff of the Medical Department is 48, all holding doctorates, two-thirds of them in medicine.

Research in clinical medicine and in fundamental laboratory medicine utilizes radiation both as a causative agent and as a label for constituents of living systems. Typical of this field is the intensive study of the relationships of radiation to blood dyscrasias. Radiation is used to induce disease for experimental study on one hand, while on the other, materials tagged with radioactivity are used to elucidate the natural history of the elements of the blood in comparison with their history under conditions of disease. It is of especial interest to note that efforts of the biochemists and the hæmatologists at Brookhaven to produce a radioactive substance which could deliver radiation selectively to the interior of cells did not bear directly the intended result, but

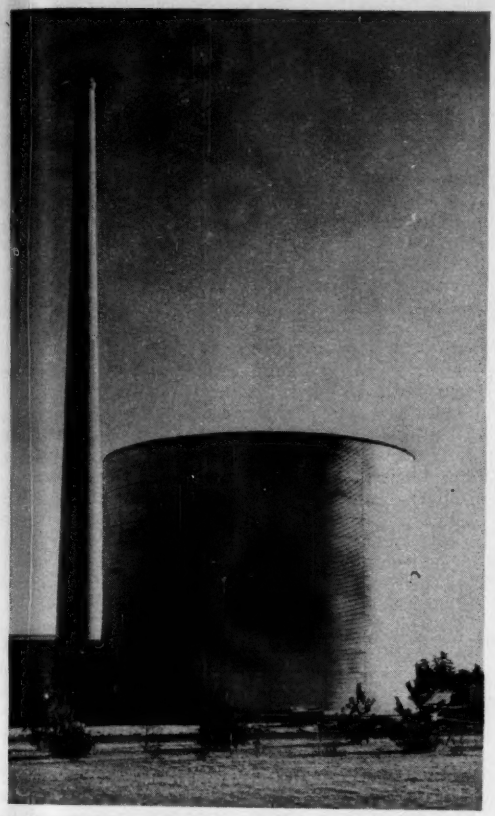


Fig. 2. Medical Research Reactor building. Cooling air for the reactor's graphite reflector is forced out of the 150-ft. stack. The steel reactor building, which is 60 ft. in diameter and 54 ft. high, is connected to the main medical research complex, where the hospital and laboratories are located

did unexpectedly provide one of the most valuable of biological tracers—tritiated thymidine—a tagged compound which immediately enters the cycle of blood constituents, appearing rapidly in large mononuclear cells of the peripheral circulation. Similarly, an attempt to develop a therapeutic application of a rapidly decaying radioisotope of manganese became an avenue opening up a broad investigation into the presence and the behaviour of trace metals in health and disease.

Much of the research programme and future planning are directed toward the special medical problems to be met in the coming nuclear age; novel tools are coming into use and are being used in various ways. Among Brookhaven's 'big machines', noteworthy use has already been made of the reactor; the high biological significance of neutrons of intermediate energy are being evaluated with a small cyclotron and a Van de Graaff accelerator; the large cyclotron has provided deuteron beams as well as rare radioactive isotopes from heavy-particle bombardment; the Hot Laboratory has searched for desirable radioactive materials of special metabolic properties. The collaboration of the scientific and technical staff at Brookhaven illustrates the co-operation which has stimulated and assisted the work of the Medical Department in its first ten years, as it grew into the present research establishment with its unique capabilities. The Medical Research Center is thus an integral unit in a scientific community of some four hundred scientists and engineers working on all the problems of the utilization of nuclear energy.

Brookhaven National Laboratory is a civilian establishment operated under the guidance of nine major universities of the eastern seaboard of the United States; they supervise its administration through Associated Universities, Incorporated, which makes contracts with the U.S. Atomic Energy Commission for supporting funds. The Laboratory is in the centre of Long Island, 70 miles east of the middle of New York City on a campus converted from the Army's former Camp Upton. In the years from its inception in 1947, as a result of the foresight of leading scientists in the new fields which research with the big machines opened up, Brookhaven has become familiar ground to hundreds of visiting and collaborating scientists from many foreign lands, all of whom have been welcomed for their contribution to the 'peaceful uses of the atom'.

The work described here is supported by the United States Atomic Energy Commission.

OBITUARIES

Prof. Arturo Duperier

Prof. Arturo Duperier, who recently died in Madrid at the age of sixty-two, was distinguished by his pioneer researches into the variation of cosmic-ray intensity with time and with meteorological conditions. He began this work in 1938 when he came to work with me and my cosmic-ray colleagues in the Physical Laboratories of the University of Manchester. Soon after the outbreak of the Second World War, when the cosmic-ray group largely dispersed for war activities, he moved his apparatus to the Imperial College of Science and Technology, London, where Prof. George Thomson gave him laboratory space. In a small room near the top of the Royal College of Science building at South Kensington, Duperier set up a cosmic-ray recording set and ran it almost continuously throughout the War and bombing, and for some years afterwards.

It was the results obtained from this long period of very precise recording of the cosmic-ray intensity which provided Duperier with the material for his analysis. His most important technical achievement was the introduction and use of multiple correlation methods for disentangling the origin of the observed variations of cosmic-ray intensity. In this way he was able to show quantitatively how the observed dependence of the cosmic-ray intensity on temperature might be an effect due to the decay of muons in the atmosphere. By careful use of meteorological data obtained from radio-sonde observations, Duperier was able to locate the average height of production of the muons as about 16 km. His method was to assume that the intensity varied both with the atmospheric pressure and with the unknown height of the muon-producing layer, and then to determine that value for the height of the layer for

muon formation which gave the largest partial correlation coefficient.

This work demanded the meticulous analysis of a great amount of numerical data, at which Duperier was exceedingly expert. To facilitate this work he pioneered the use of business accounting machines for the analysis of cosmic-ray data. Later he refined his analytical method by introducing a three-term regression formula, and by this means obtained some indication—but no final proof—of the effect on the cosmic-ray intensity at sea-level of the pion decay in the upper atmosphere. This last work was carried out at Birkbeck College, where he moved soon after the War. In 1945 he gave the Guthrie Lecture to the Physical Society of London on the "Time Variations of Cosmic Rays".

Duperier was well known to cosmic-ray workers all over the world and regularly attended the biennial meetings of the International Cosmic Ray Conference, under the auspices of the International Union of Pure and Applied Physics, which has met since the War in Poland, India, France, Mexico and Italy. He will be greatly missed at the next meeting, planned for this summer in Moscow.

In 1951 Duperier returned to Spain, where he resumed his professorship in the Faculty of Science in the University of Madrid. The loss to his country of the most distinguished Spanish atomic physicist of his generation is indeed great. He was awarded posthumously the Juan March Foundation prize of 500,000 pesetas for his outstanding scientific work.

Throughout Duperier's thirteen years in England, I was in close touch with his work, and came greatly to admire him not only as a scientist but also as a man of unbending rectitude of character. It is thus both as a fine scientist and as a close personal friend that I mourn his death. P. M. S. Blackett

Prof. Pierre Fauvel

Pierre Fauvel, professor in the University of Angers, who died on December 12, was one of the greatest systematic zoologists. A specialist on Polychaeta, a group with which his name will for ever be associated, Fauvel was supreme in his field. No one who preceded him and none of his contemporaries approached the brilliance of his systematic interpretation.

Fauvel was born at Cherbourg on October 8, 1866. Throughout the greater part of his professional life he was on the staff at the Catholic University of Angers, as professor of zoology for fifty-three years, with a term of office as dean of the Faculty of Science from 1942 until 1947.

In the early stages of his career, 1894–1909, Fauvel worked on human physiology, publishing papers on digestion and uric acid secretion, and on general marine biology. In the latter field he carried out research on the larval development of *Arenicola*, on the circulation in Amphictenidae, on otocysts and on nephridia. For his doctorate of science he published observations made on the Ampharetidae. During 1910–20, however, the trend towards systematics becomes more apparent, and Fauvel's first major work in this field was on collections of polychaetes made in the Mediterranean and Atlantic during cruises of *l'Hirondelle* and *Princesse Alice*, under the direction of Prince Albert of Monaco. There followed papers on collections from the Falkland Islands, Australia, West Africa and Madagascar in this period, and all indicated that a new and brilliant figure had arisen.

With the publication in 1923 and 1927 of his two volumes in the "Faune de France" series, Fauvel's position as the outstanding authority on polychaetes was established. He provided in these works a practical method of identifying almost 900 species, his sedulous preparation of keys, in particular, being of the greatest value. In the genera *Harmothoë*, *Nereis* and *Serpula*, numerous species, previously a jumbled assortment of specimens and populations, were reduced to a series of well-defined natural groups as clearly delineated as available knowledge allowed. For those most difficult of families, the Phyllodocidae and Syllidae, his consideration of the species concept brought order out of confusion. This same ability to define species and clarify taxonomic problems is evident in all the families, so that these volumes have been in continual use in practical marine courses throughout the world since publication, and will probably not be replaced, even by local faunal publications, for years to come. Marine ecologists and physiologists everywhere praise their value and no specialist would consider approaching a problem concerning polychaetes before first noting what Fauvel had to say. In addition, these two works represent models of how the perfect faunal study should be presented, and their extension to other groups (and other areas for Polychaeta) is of paramount importance.

In 1932 Fauvel's work on the polychaetes from the Indian Museum in Calcutta was published, and this also is an invaluable guide to the student and specialist. It was the first comprehensive publication on polychaetes from subtropical and tropical waters, and has been the basis of all later diagnoses of the Indo-Pacific fauna.

Through experience with populations from widely separated regions, Fauvel was able to demonstrate the cosmopolitan nature of many species and the unique endemic elements in particular areas. He was the first to recognize the occurrence in Europe of *Mercierella enigmatica* as an invasion, possibly from India, of a species capable of breeding in waters of low salinity. Since then, repeated records of this polychaete from harbours in western Europe have indicated how damaging the effects of its growth might be.

Honours, richly deserved, were paid to Fauvel by his native France; they included: Chevalier de la Légion d'honneur; Lauréat de l'Institut, Prix Savigny de l'Académie des Sciences; Membre de la Société Zoologique de France; Membre Correspondant du Muséum d'Histoire naturelle de Paris. It is indeed with pride that systematic zoologists can point to the career of Pierre Fauvel, for he always upheld the finest traditions of learning in pursuing his chosen subject to the utmost of his ability, and he gave to zoology some of the finest products of scholarship published during his life-time.

Norman Tebble

Dr. Herbert Hunter

Dr. Herbert Hunter died on February 21 at Meldreth, near Cambridge, at the age of seventy-six, after a long and productive working life, during which he had made outstanding contributions to plant breeding, to agriculture and to the malting and brewing industries. He will always be remembered for his activities in these fields, and also as a dedicated research worker and student of his subject who kept his interest in his work up to the time of his death.

Hunter graduated from the University of Leeds, of which he was a D.Sc., and started his researches into barley breeding in the early 1900's. This early work was conducted in Ireland, where he was head of the Plant Breeding Division, Department of Agriculture, Dublin, while he also became the first director of the Stormont Plant Breeding Station in Northern Ireland. Later, during 1936–46, Hunter was director of the Cambridge University Plant Breeding Institute. When in Ireland he bred the hybrid barley variety, Spratt-Archer, which was afterwards to make his name when it became one of the most successful varieties of cereals ever bred for Great Britain. It was introduced into cultivation in 1914, and has only been superseded as a standard malting barley in recent years. Hunter went to Cambridge in 1924, and he there bred two more varieties of barley—Earl and Camton—and a winter oat variety named Picton. All these varieties found a place in British agriculture and received official recommendation for farmers. Hunter's contributions gave him an established position with Sir Rowland Biffen and Dr. E. S. Beaven as successful pioneer plant breeders exploiting the new genetic knowledge.

This work ensured the recognition of Hunter's name nationally and internationally, but he also acquired a great reputation as a writer. He started contributing scientific papers and articles as early as 1908, and maintained a steady output throughout his life. He was an easy, lucid and fluent writer, and found an outlet for his talent in writing books, of which he had a number of successes to his credit. He wrote a standard work on oats, and one on barley in the 1920's, while in 1931 he edited a new edition of Baillière's "Encyclopædia of Agriculture" (with H. Martin Leake), while his most recent contributions were "Crop Varieties" (1954) and "The Barley Crop" (1956).

One of Hunter's great interests was the work of the National Institute of Agricultural Botany, to which he gave most valued service as a member of Council for twenty-one years, as acting director during the Second World War, and as chairman of Council for three years. For his services he was elected a vice-president of Council, a signal recognition of the way in which he guided the Institute's work and activities and established its present important relationships with agriculturists and commercial interests in the seed trade. Hunter's great tact, wisdom and kindliness were all instrumental in accomplishing this work so successfully, and those who worked under him in both Institutes which he directed were grateful for the experience.

In 1911 Herbert Hunter married Rosa Wise, of Springfield, Ohio. She died in 1945, and Hunter married in 1951 Mrs. Blanche Metters, widow of J. D. Metters. He is survived by his widow and two daughters of his first marriage. G. D. H. Bell

Dr. Alexander M. Cockburn

On February 28 Dr. Alexander Murray Cockburn, senior lecturer in geology in the University of Edinburgh, died at the age of fifty-seven.

Dr. Cockburn graduated with first-class honours in geology at Edinburgh in 1924. After a season with the Geological Survey of Canada, and some time spent in studying volcanic phenomena in Hawaii, he returned to his native city to take up postgraduate research as a Falconer Fellow of the University. In 1927, with John Mathieson, Dr. Cockburn carried out the first detailed topographic survey of the St. Kilda group of islands, and in the following year he completed a geological study of these islands, which was published in the *Transactions of the Royal Society of Edinburgh* in 1935, and remains a distinguished contribution to our knowledge of Scottish geology.

Dr. Cockburn was awarded the degree of Ph.D. in 1929, and was elected a Fellow of the Royal Society of Edinburgh in 1935. He began his long service as a member of the staff of the University of Edinburgh in 1931, and passed successively through the positions of assistant and curator of the museum, lecturer, and senior lecturer. In 1942, he took over from Prof. T. J. Jehu the courses in stratigraphy, and since then he was responsible for the formal stratigraphy courses in the Department. However, he never lost his early interest in vulcanology, and he made a comprehensive study of the volcanic rocks of the Pentland Hills. It is most unfortunate that his early death should have prevented him from publishing more than a small part of this work.

From 1951 onwards Dr. Cockburn acted as director of studies in pure science, and he was a member of the Senatus Academicus of the University of Edinburgh from 1956. A prominent Fellow of the Edinburgh Geological Society, he served as honorary secretary during 1939–44, and was elected president in 1958.

These few details of Dr. Cockburn's career can reveal little of the enormous debt which is owed to him by his Department and by all who have been associated with it. He combined a complete and unselfish loyalty to his subject and his Department with a deep and stimulating understanding of the personal problems of his students. He was a most able and conscientious teacher, both in the lecture room and in the field, and he carried a considerable administrative load. One of his great interests was the geological museum, and to this, and to the care of the Grant Institute building, he devoted a great deal of time and skill. Recently he has played a large part in the planning associated with a new building programme, and in the change from a three-year to a four-year honours course in geology. All the students who came under his care will remember with gratitude and affection the help and advice which he gave so readily, and his unfailing kindness, wisdom and good humour. All who were fortunate enough to know him feel a tragic sense of personal loss. F. H. Stewart

Dr. S. Bekku

By the death on September 19 last in Tokyo of Dr. S. Bekku, Japan has lost one of her distinguished electrical engineers.

Dr. Bekku was born at Ueno, Tokyo, in 1893, and he graduated in electrical engineering from the Tokyo Imperial University in 1917. After graduation he pursued advanced studies in the Electro-Technical Laboratory on electrical power transmission and high-voltage engineering. It was at this stage that Dr. Bekku had the opportunity of going to the United States and working with the Westinghouse Electric Company under the direction of Dr. C. L. Fortescue. In 1927, Dr. Bekku received the degree of doctor of engineering from the Tokyo Imperial University, and in 1928 he was appointed to a professorship in the Tokyo Institute of Technology. Here he lectured on transmission and high-voltage engin-

eering. In 1931, Dr. Bekku took up an appointment with the Sumitomo Electrical Industries, where he was responsible for important research and development work in the field of electrical power transmission and distribution.

During the period up to the outbreak of the Second World War, Dr. Bekku published papers dealing with measurements on overhead transmission lines, unbalanced alternating current circuits, concentric cables and power capacitors.

In 1952, Dr. Bekku was elected president of the Institution of Electrical Engineers of Japan. In 1956, the Central Power Research Laboratory, a research association which serves the nine major electricity supply organizations in Japan, appointed Dr. Bekku its director and he was responsible for work on the development of the 380-kV. transmission system.

Dr. Bekku took great pleasure in the maintenance of his contacts with electrical engineers and physicists in the United States. He had travelled extensively in Europe and America and he enjoyed climbing and skiing. Dr. Bekku is survived by his widow, two sons and two daughters.

NEWS and VIEWS

The Rockefeller Foundation : New Officers

Dr. J. George Harrar has been elected vice-president of the Rockefeller Foundation. Dr. Robert S. Morison has been elected director for medical and natural sciences, a programme merging the biological, medical and public health interests of the Foundation. Dr. John C. Bugher is to be consultant on nuclear energy and as a principal officer will assist all programmes with respect to the impact of nuclear energy. Dr. Albert H. Moseman has been appointed deputy director for agricultural sciences, the new designation of the former agricultural programme. Dr. Lindsley F. Kimball continues as executive vice-president and Dr. Warren Weaver as vice-president for the natural and medical sciences.

Dr. Harrar joined the Foundation in 1943 as local director for the agricultural operating programme in Mexico. He became deputy director for agriculture in 1952 and director in 1955. Dr. Harrar is also serving in a special capacity as director of the National Academy of Sciences Survey on Education, Science and Technology in Africa south of the Sahara. When he joined the Foundation he was the sole staff of its agriculture programme. Established with Dr. Harrar's arrival in Mexico in 1943, this agricultural programme now has operating units in Mexico, Colombia, Chile and India, an extensive international fellowship and scholarship plan, and a grants programme in support of agricultural research and education. Approximately 6,270,000 dollars was appropriated last year by the Foundation in support of these three types of activities.

Dr. Morison was formerly director for biological and medical research in the Foundation. A graduate of Harvard University and of the Harvard Medical School, he taught in the latter before joining the Foundation in 1944. Dr. Bugher, a member of the Foundation's staff since 1943, was given leave of absence to serve for four years as director of the Division of Biology and Medicine of the U.S. Atomic Energy Commission. He returned to the Foundation in 1955 as director for medical education and public health. He represented the Atomic Energy Commission officially at the Geneva Conference on the Peaceful Uses of Atomic Energy in 1958 and is a member of the Commission's advisory committee on biology and medicine. Dr. Moseman joined the Foundation in 1956. He was previously director of crops research of the agricultural research service of the U.S. Department of Agriculture.

National Science Foundation Office of Social Sciences

The National Science Foundation has recently established an Office of Social Sciences with Dr. Henry W. Riecken as its head. The Foundation has also appointed an Advisory Committee for the Social Sciences to advise on programmes and policy, the members of which are : Dr. Leonard S. Cottrell, social psychologist, Russell Sage Foundation ; Prof. Fred Eggan, professor of anthropology, University of Chicago ; Dr. John Gardner, president, Carnegie Corporation ; Dr. Pendleton Herring, president, Social Science Research Council ; Prof. Joseph Spengler, professor of economics, Duke University ; Prof. S. S. Wilks, professor of mathematics, Princeton University ; and Dr. Dael Wolfle, executive secretary, American Association for the Advancement of Science. The National Science Foundation intends to support basic research in the social sciences, since such support is invaluable in assisting social scientists to improve their research techniques, to accumulate fundamental knowledge about human behaviour and society, and to develop sound theoretical bases for further inquiry.

British Scientific Attaché in Moscow : Mr. D. A. Senior

Mr. D. A. Senior, of the Royal Naval Scientific Service, has been appointed to the newly created post of scientific attaché to the British Embassy in Moscow ; he will advise the British Ambassador, Sir Patrick Reilly, on scientific matters and report on Soviet scientific and technical development in the civil field. Mr. Senior, who is thirty-five, will hold the rank of senior principal scientific officer and will be on the staff of the Department of Scientific and Industrial Research. Mr. Senior attended Batley Grammar School, Yorks, where he won an open scholarship to Trinity College, Cambridge. He graduated at Cambridge in 1943 with first-class honours in mechanical sciences (with distinction in electronics). He also won the Charles Lamb Prize in electrical engineering. In 1946 he shared the John Wimbolt Prize in civil engineering. From Cambridge he entered the Naval Construction Research Establishment at Rosyth, where he worked on high-speed photography. Since 1950 Mr. Senior has been at the Admiralty Research Laboratory, Teddington, mainly investigating problems of instrumentation and combustion. Recently, he carried out a study of acoustic

methods of fish-finding. He has also been studying combustion on a part-time basis in the Chemical Engineering Department of the Imperial College of Science and Technology, London. Mr. Senior has a good knowledge of French, German and Russian. He is married and has three sons.

Peabody Museum, Yale : Prof. C. O. Dunbar

PROF. CARL O. DUNBAR, who will retire from the directorship of the Peabody Museum at Yale on July 1, will then have served thirty-nine years on the faculty of Yale and sixteen years as director of the Museum. Prof. Dunbar graduated at Kansas in 1914 and obtained his Ph.D. at Yale in 1917. He was appointed assistant professor of historical geology at Yale in 1920 and then held various other geological appointments at the same University until he became curator of invertebrate palæontology and eventually director of the Peabody Museum. Dunbar is a palæontologist of international reputation and the author of many papers, and he has been editor of several scientific journals. His chief work concerned brachiopods and the Permian foraminifera. He is a Fellow of the Geological Society of London. His distinguished directorship of the Peabody Museum has been characterized by a wise and progressive policy.

Dr. S. Dillon Ripley

DR. S. DILLON RIPLEY, a member of the faculty of Yale since 1946, has been appointed director of the Peabody Museum in succession to Prof. Dunbar. Prof. Ripley is an outstanding authority on birds, particularly those of the Far East, and is president of the International Council for Bird Preservation, an organization with sections in forty-five countries. Ripley graduated at Yale in 1936 and took his Ph.D. in zoology at Harvard in 1940. He served in the Second World War with the Office of Strategic Services, and was appointed to Yale as lecturer and assistant curator of zoology in 1946 and became associate professor in 1955. He has spent the major portion of the time outside the United States in the study of bird life. In several Asiatic areas he has been the first white man seen by the local population. He is also a distinguished writer and his works combine philosophy and literary quality with the recording of scientific facts. With such a background of experience the future of the Peabody Museum and its collections is assured.

The Argus High-altitude Geophysical Experiments

DR. ALAN T. WATERMAN, director of the National Science Foundation, and Dr. Detlev W. Bronk, president of the U.S. National Academy of Sciences, have issued a joint statement referring to the Argus high-altitude geophysical experiments last August and September. Reduction, analysis and evaluation of the observations have been going on, in order to interpret those of scientific significance. Plans are well advanced for the orderly distribution to the world's scientists of appropriate scientific information, as a part of America's participation in the International Geophysical Year. As scientific papers are completed, publication will proceed through the appropriate scientific journals. In addition, plans have been made to summarize the scientific results in the course of one of the four symposia to be held at the annual meeting of the U.S. National Academy of Sciences during April 27-29.

Atomic Power Station for Japan

THE Japan Atomic Power Company has announced in Tokyo that on April 3 it had issued a letter of intent to the General Electric Co., Ltd., of England, for the construction of a 150-MW. atomic power station at Tokai-Mura, some seventy miles north-east of Tokyo. This order will be worth in the region of £20,000,000 and marks an important step forward for the General Electric Company, which has been reorganized into three main groups with the view of giving greater drive to its main activities, particularly the development of its heavy engineering and nuclear energy side. The success achieved in Tokyo has been accompanied by several other large engineering orders during the past month, including an order for two 200-MW. turbo-generator sets for the Kincardine station of the South of Scotland Electricity Board, valued at about £4,000,000, and an order for a complete ore preparation plant for the Redbourn Works of Richard Thomas and Baldwin, Ltd., valued at about £1,250,000.

Popularizing Astronomy

THE International Geophysical Year and the launching of artificial satellites have led to a greater public awareness of scientific developments. This has created a need for the supply of accurate information in a more systematic manner than is possible in the average newspaper and with less technical detail than is appropriate even in an avowedly popular scientific magazine. The series of twelve articles which Dr. R. A. Lyttleton is contributing to the *Illustrated London News* is therefore especially welcome. Under the general heading, "The Universe at the Beginning of the 'Space Age' ", Dr. Lyttleton has written twelve articles. The first of these appeared on February 21, and the others are being published weekly in subsequent issues. The facilities provided by the *Illustrated London News* through their artist, Mr. G. H. Davis, are being used to great advantage in illustrating the articles. The first two articles dealt with the Earth and terrestrial meteorite craters. The next six deal with the Sun, planets, comets and the origin of the solar system, and the last four articles will deal with the universe of stars and galaxies.

Navigational Aids

IN reply to questions in the House of Commons on March 18, asking for a statement regarding the recent meeting of the International Civil Aviation Organization at Montreal, the Minister of Transport and Civil Aviation, Mr. H. Watkinson, said that, in the Government's view, present standards of safety in congested air space could not be maintained with the use of VOR-DMET, the United States system of short-range navigational aid for aircraft, without seriously reducing the efficiency of air traffic control. Although twenty of the thirty-seven countries represented at the Conference voted for the adoption of this system, the British Decca system was the only navigational aid at present available which met the requirements of the International Federation of Air Line Pilots Association for an accurate and reliable short-range navigational aid based on the area coverage system, and designed to provide pictorial presentation of navigational information to the pilot in the cockpit. In the Government's view the Conference had not based its considerations on sound and objective grounds and the Government would continue to press for a more satisfactory

solution. Mr. Watkinson hoped that the final decision, which vitally affected air safety, would be taken on proper technical grounds and not on grounds subject to pressure, national prestige or any other consideration than that of the safety of passengers in the air. Mr. Watkinson deprecated the lobbying that had occurred and agreed that, as neither system was fully proved, it would be wise to leave the decision for a period for further technical experience to be accumulated.

Research Associations

In answering questions regarding the British Hat and Allied Feltmakers Research Association in the House of Commons on March 17, the Parliamentary Secretary to the Ministry of Works, Mr. H. Nicholls, said that the Department of Scientific and Industrial Research had recently rejected the idea of a special grant to the Association in view of its development of No. 11 antishock felt in the production of protective caps for motorists and other road users. The Road Research Laboratory had been consulted by a hat manufacturer and had suggested that felt might be used in protective hats, and the manufacturer and the Research Association had co-operated with the Laboratory in selecting a quality, density and thickness of felt for antishock protection; but he stressed that such felt hats were no substitute for the motor cyclists' crash helmets, although they undoubtedly offered some protection. In reply to a further question regarding facilities in the Department of Scientific and Industrial Research for assisting in the choice of scientific instruments, Mr. Nicholls said that an instrument inquiry service is provided at the British Scientific Instrument Research Association and is largely financed by a contribution from the Association and an equal grant from the Department of Scientific and Industrial Research. Inquiries were at present running at the rate of 2,200 a year. The Research Association had a working party on instrument design which, inter alia, reviewed the specification, price and appearance of foreign instruments, and other research associations as well as the Department's research stations also designed new instruments.

Memorial Meeting to Prof. F. Joliot-Curie

A memorial meeting for Prof. Frédéric Joliot-Curie was held at Friends House, Euston Road, London, on January 22. It was attended by many scientists invited by the Association of Scientific Workers and by the National Committee of Science for Peace. The chair was taken by Prof. C. L. Oakley, president of the Association of Scientific Workers, and the speakers were Prof. C. F. Powell, Dr. Pierre Biquard and Prof. J. D. Bernal.

Prof. Powell outlined the contribution made by Joliot and his wife, Irene Curie, towards the discovery of the neutron, leading to the discovery, in 1934, of artificial radioactivity, for which in 1935 he received the Nobel Prize for Chemistry. Dr. Pierre Biquard, secretary-general of the World Federation of Scientific Workers and a close friend of Joliot's, described the joy Joliot had expressed to him at having been able to add to the contribution of French science to man's knowledge. Dr. Biquard also spoke about the part played by Joliot in the French Resistance during the Nazi occupation, when he refused to leave his country and stayed to help defend it after arranging for the valuable stock of heavy water to be brought safely to Britain. Prof. Bernal described Joliot's work on the international level. In the 1930's he had been a member of the Anglo–French Scientific Committee which had laid the basis for the foundation in 1946 of the World Federation of Scientific Workers, of which he became the first president. He had also taken an active part, as a representative of France, in the foundation of Unesco. Later, in 1949, Joliot was chosen as president of the newly formed World Council of Peace, a post which he held until his death.

International Journal of Radiation Biology

International Journal of Radiation Biology and related studies in Physics, Chemistry and Medicine is the full title of a new journal, the first issue of which is dated January 1959 (1, No. 1. Pp. 104. Subscription per volume (4 issues) 75*s*., post paid; price per part 20*s*. plus postage. London: Taylor and Francis, Ltd., 1959). The editor is Dr. W. M. Dale, of the Department of Biochemistry, Christie Hospital and Holt Radium Institute, Manchester, and to assist him there is a large editorial board with distinguished representatives from Europe, the United States, Australia and Japan; regrettably, the U.S.S.R. is not represented. The first paragraph of the editorial gives a concise description of its aims: "In this new journal we propose to bring together all interests concerned with the biological effects of ionizing radiations. The field we hope to cover will not, however, be a narrow one, for it will embrace any discipline—medicine, physics and chemistry, for example—which has a bearing on our main theme".

The introduction of a new journal invariably arouses mixed feelings. We experience apprehension at the thought of having to add yet another publication to our check list, but on referring to this list, we find, rather surprisingly, that this new journal is the first primarily concerned with the important and growing subject of radiation biology. In the past there has been a tendency to regard radiation biology either as an appendage of radiotherapy or as a highly specialized version of applied physics. These views are completely unjustified and the practitioners of medicine and physics are necessarily joined by radiation chemists, biochemists, cytologists and geneticists in a combined attack upon a highly complex set of problems. The ten original articles in the first issue illustrate this clearly and embrace each of the above-mentioned disciplines. The international scope is revealed by the fact that these ten articles originate from no fewer than seven different countries. Articles may be submitted in English, French or German, accompanied by short summaries in all three languages; all articles in the first issue are, in fact, in English.

Publications in Microform

The problem of providing adequate storage space for the ever-increasing number of publications in science and technology is one that constantly haunts librarians. For journals which may be consulted infrequently and for books of historic importance some form of microform storage appears to be inevitable. The making of the most suitable types of microform materials itself raises problems of mechanics and storage and, for these and other reasons, librarians will welcome the enterprise of Micro-Methods, Ltd., of East Ardsley, Wakefield, Yorks, which has issued extensive lists of journals that have already been issued in micro-card, micro-film or micro-fiche form. Other lists contain details of rare and out-of-print books which have also been produced

in microform. Among the many journals available are the *Journal of the Marine Biological Association* (1887–1913); the *American Journal of Psychiatry* (1921–50); the *Japanese Journal of Astronomy and Geophysics* (1922–47); *Jahrbuch über die Fortschritte der Mathematik* (1868–1944); and the *English Historical Review* (1886–1910). Rare books available in microform include Brongniart's "Histoire des Végétaux Fossiles", published in 1828, and Thumberg's "Flora Japonica", published in 1784. A series of theses from the Faculty of Metallurgy at the University of Sheffield is at present being prepared in microform. The charges of Micro-Methods, Ltd., appear to be reasonable and the company would welcome inquiries about the preparation of any publications in micro-form.

Anglo-American Library Associations

In Library Association Pamphlet No. 19, Mr. R. D. MacLeod gives a historical note on the beginnings of the Anglo-American Library Associations (pp. 15. London: Library Association, 1958. 2*s*.), which indicates that even eighty years ago the advantages to be reaped by co-operation were already evident. There is a useful bibliographical note, and apart from its historical interest the paper records an episode to which some parallel could be found a generation later in the matter of chemical abstracting.

University Library, Leeds

The annual report of the Librarian of the University of Leeds for the session 1957–58 (pp. 12. Leeds: Brotherton Library, The University, 1958) records accessions of 15,951 volumes, 3,306 pamphlets and 27,989 periodical parts, an increase of 11 per cent, bringing the holdings to 428,570 volumes and 173,311 pamphlets. Loans to readers totalled 101,220 and inter-library loans 6,041, the number of books lent increasing by 42 per cent. Keeping the library open until 10 p.m. proved an immediate success and has now been introduced on a permanent basis, but there was insistent pressure on seating accommodation and the staff position caused anxiety. The Nuffield pilot survey of library use was completed at the end of 1957 so far as the collection of material was concerned.

City of Leicester Museums and Art Gallery

The annual report of the Museums and Art Gallery at Leicester for the year ending March 31, 1958 (pp. 35+4 plates. Leicester, 1958), which is well illustrated, records difficulties in recruiting senior staff for museum work and also the completion of the reconstruction work on the roof of the main building. This had necessitated the re-designing of the natural history galleries on the first floor. The Schools Department has been able to increase the number of loans of museum material to schools, but the demand still outstrips the supply. A record was achieved in the number of temporary exhibitions shown, and the Saturday evening lectures maintain their appeal.

The Parliamentary and Scientific Committee

The annual report for 1958 of the Parliamentary and Scientific Committee (pp. 26. 1959), presented to the annual general meeting on February 18, includes a list of addresses and discussions held during the year. These dealt with the present and future position of the universities, with special reference to scientific and technological education and research; space satellites; latest developments with regard to the use of thermonuclear energy at power stations, with special reference to *Zeta*; the five-year plan for the development of China's agricultural resources; the work of the Department of Scientific and Industrial Research; veterinary science, its progress and application in Great Britain; international science; exploration in Antarctica, with reference to the International Geophysical Year; the research and development background to the railway modernization programme; and the medical problems of living in the nuclear age. Representatives of the Committee's Technical Education Sub-Committee discussed with the Minister of Education the question of future finance for colleges of advanced technology, and the Committee has also made representations on the appointment of a person with no legal or scientific qualifications as comptroller-general of the Patent Office, and is seeking further information about the disbanding of the Microbiology Group at the National Chemical Laboratory. A memorandum was submitted in May to the Pritchard Committee set up to consider the rating of charities and similar organizations including scientific societies, and in moving the adoption of the annual report on February 18, the chairman, Mr. R. Fort, said that the Committee and the Royal Society were the only organizations from which the Pritchard Committee took oral evidence; its report is expected early in 1959. Mr. Fort also referred to the way in which the Parliamentary and Scientific Committee could promote the integration of scientific disciplines, which he thought was the most probable line of scientific advance, and to the importance of maintaining varied sources for grants for scientific work.

Southern Regional Council for Further Education

The eleventh annual report of the Southern Regional Council for Further Education, covering the year 1957–58 (pp. 36. Reading: October 1958), records rapid progress in the development of technical education and steady growth of courses at all levels throughout the region. Regional procedure for considering proposals to establish new advanced courses operated smoothly and satisfactorily, while the record of developments in colleges in the area continues to be impressive. The Ministry of Education has now taken over from the Regional Council the annual publication of lists of sandwich courses available throughout Britain: List 182 details 263 courses being held in the session 1958–59, an increase of 65 on the previous session. Stress is laid on the importance of financial assistance in the development of such courses and on the need both for financial assistance from industry for its own employees and of co-operation between authorities and industry for others. Progress is reported in the establishment of formal and informal links between technical colleges and secondary schools in the area, and the Council has asked constituent authorities to agree to a new basis for arranging courses for technical teachers. At the request of the Council, the Regional Academic Board has begun a survey to assess the needs of industry in relation to the development of technical colleges, and attempts have been made to form an area group of the British Association for Commercial and Industrial Education. The Board has secured the presence of heads of grammar schools as members of advisory committees. A list of research projects and investigations carried out since 1955 in technical colleges in the region is included.

Science, Philosophy and Religion

THE Eddington Memorial Lectures have come to occupy a special place in the scheme of things, because they enable men of distinction to illuminate some particular facet of the working of that great mind. In his recent lecture entitled "Science, Philosophy and Religion" (pp. iv+31. Cambridge: University Press, 1959. 4*s*. 6*d*. net), Sir Russell Brain shows how the analysis of man, science and revelation, man and mechanization, perception and knowledge fit into the main stream of Eddington's thought; and this because the author is dealing with someone who was all at once Quaker, mystic, mathematician, astronomer and metaphysician. If one lesson emerges clearly from pondering upon Eddington's philosophy, it is that a new concept will be needed to enable us to explain total behaviour in a way which transcends individual components. This is well said for biological systems, but it (or something very like it) is becoming essential for inanimate phases, too, as witness the present discontents with 'classical' quantum theory. Ratiocination will try its hardest, though maybe these regions form part of the unseen world to which, as Eddington says, the human spirit must turn.

Design for Tropical Living

GROWING interest in the effects of environmental factors on life in tropical regions is to be found in the number of conferences and specialist meetings which have been held during the present decade. In 1951 an informal meeting was held in London to discuss co-operative research on building problems arising in hot climates. Unesco has organized two symposia, one held in New Delhi in 1952 to study the application of scientific principles, and one in Uganda in 1955 dealing with geographical factors in relation to the design of tropical buildings in the development of tropical Africa. The United States Building Research Advisory Board arranged a conference in Washington in 1953 on building in hot-humid and hot–dry climates, while in the same year, ecological problems arising from technical developments in tropical and sub-tropical countries were considered at an informal seminar held at Cambridge.

In 1955 medical and other aspects of African housing were considered at a conference under the ægis of the East African Advisory Committee for Medical Research in Nairobi. The Australian Academy of Science held a symposium in Brisbane in 1956 on man and his animals in the tropics, while in September 1958 the Scientific Council for Africa South of the Sahara arranged a specialists' meeting at Lagos on environmental factors affecting the physiology of man. The findings of these and other investigations were brought together during an admirable symposium arranged by the Natal Regional Research Committee of the South African Council for Scientific and Industrial Research and the University of Natal at Durban on October 18, 1957. The papers dealt with the climatic environment, deterioration of materials, physiological aspects of design for tropical living, psychological aspects of life in a warm climate, town and regional planning, climatic control by building, design, air conditioning in the tropics, and architecture in the tropics. These, and the ensuing discussion, have been published in a report (Symposium on Design for Tropical Living. Pp. vii+222. Pretoria, 1958) prepared by the National Building Research Institute of the South African Council for Scientific and Industrial Research.

Report of the Smithsonian Institution

THE annual report of the Board of Regents of the Smithsonian Institution for the year ended June 30, 1957 (cf. *Nature*, **181**, 1709; 1958), has now been published as a bound volume (pp. x+499+74 plates. Washington, D.C.: Government Printing Office, 1958. 4.50 dollars) with a general appendix, including memoirs and reports of investigations made by members of the staff of the Institution or collaborators. Among those not previously published or not easily available may be mentioned E. B. Roberts's review of the United States Coast and Geodetic Survey, 1807–1957; Prof. T. Gold's James Arthur lecture, "Cosmic Rays from the Sun"; F. L. Whipple's "Meteors"; J. M. Chamberlain's "The Development of the Planetarium in the United States"; R. Lee's "Jet Streams"; E. B. Leopold and R. A. Scott's "Pollen and Spores and their Use in Geology"; Prof. R. E. Crist's "The Land and People of the Guajira Peninsula"; M. C. Leikind's "Aniline Dyes—Their Impact on Biology and Medicine"; and T. D. Stewart's "Stone Age Skull Surgery". A limited number of reprints of the papers are available on application to the Editorial and Publications Division, Smithsonian Institution.

Rand Corporation Publications

THE Rand Corporation has presented to the Science Museum Library, as a national deposit collection for Great Britain, a set of nearly 1,000 of its research publications. These range through mathematics, physics, electronics, aeronautics, economics, and the social and political sciences. They are now available for loan to all registered borrowers from the Science Museum Library, and to anyone as photocopies through the Science Museum Library Photocopy Service. The Index to the Rand Corporation Publications is held at the Science Museum Library, but is not for loan. Copies of the Index have also been deposited in the larger university libraries of Great Britain.

Design of Office Chairs and Tables

GENERAL principles which may be applied to the design of chairs and tables can be discerned in the results of a number of researches undertaken during the past few decades in several related biological fields. Studies of anatomical structure and function have given information as to where support for the body should or should not be provided. Clinical evidence demonstrates the results of insufficient attention to body mechanics. Studies of the times at which different muscle groups come into play and become fatigued in various activities, and the extent of their action in different positions, indicate the relative physiological efficiency of different working postures. Many anthropometric surveys have now been made with application to a wide variety of practical, industrial and military problems.

From these studies Dr. W. F. Floyd and Dr. D. F. Roberts have selected the findings relevant to the dimensions and design of office chairs and tables and summarized them in a report published by the British Standards Institution (Anatomical, Physiological and Anthropometric Principles in the Design of Office Chairs and Tables. B.S.3044, 1958. 5*s*. net). Among the factors considered are: posture, supporting the trunk weight, seat height, seat depth, seat width, tie rails, trunk stabilization, the backrest and table height. The report contains a biblio-

graphy giving details of previous studies in this and other related fields.

Fishing Gear of England and Wales

THE Ministry of Agriculture, Fisheries and Food has recently published a revised edition of the late F. M. Davis's "An Account of the Fishing Gear of England and Wales" (Fishery Investigations, Series 2, Vol. 21, No. 8. Pp. v+165. London: H.M. Stationery Office, 1958. 35*s*. net). Although a new edition of this standard reference work, now twenty-two years old, is to be welcomed, one can only regret that the opportunity has not been taken to bring it thoroughly up to date. Far too much of the book is taken up with descriptions of methods which are of interest only to the historian of fishing—methods which could equally well be found in a library copy of the earlier editions. This leads to an imbalance in the presentation. Thus the description of the Larsen trawl occupies no more space than that of the older (and much simpler) stownet which it has now almost entirely superseded, while the largely derelict fishing weirs, now only a memory to most fishing communities, receive fuller treatment than either. Despite, however, the resulting distortion of the overall picture of British fishing methods, the work remains an invaluable compendium of fishing practice around the United Kingdom.

The Wonderboom

THE famous wonderboom tree of Pretoria has been described as "the most remarkable example of its species in Africa and a National Monument". This magnificent 'tree' nestles against the sheltered north slope of the Magaliesberg range, a few miles north of Pretoria and close to the River Apies. The tree is an evergreen wild fig, *Ficus pretoriae* Burtt-Davy. There are many examples of this species in the district, especially along the northern base of the Magaliesberg range, and fine specimens have been recorded as far west as Marico. The best-known individual tree is the klein wonderboom, which is to be found a quarter of a mile to the west of Wonderboom Poort. The wonderboom is remarkable because of its mode of spread; no other example of this species has been found which has attained such considerable dimensions. The spread resembles that of the strawberry plant, the branches taking root with new trunks growing up until the single tree looks like a miniature forest. In the klein wonderboom thirteen 'individuals' have arisen from the original trunk. From north-north-east to south-south-west the diameter of the tree is about 170 ft. and from east to west about 145 ft. It is at least 74 ft. high. This famous banyan tree is described in a special issue of the *Transvaal Fauna and Flora* (No. 7; 1956), which is devoted entirely to the Wonderboom Nature Reserve.

Respiration of Excised Tomato Roots

IN a further contribution to our knowledge of the carbohydrate nutrition of tomato roots grown in pure culture, D. R. Morgan and H. E. Street have examined the sugars, and sugar phosphates and alcohols, which may serve as respiratory substrates (*Ann. Bot.*, N.S., 23, 89; 1959). The respiratory responses of substrate-depleted excised root tips to a range of sugars, sugar phosphates and sugar alcohols were determined by measuring oxygen uptake by the direct method of Warburg. Sucrose, dextrose and lævulose are the only sugars which promote a high level of oxygen uptake. The effects of azide and dinitrophenyl on the oxygen uptake promoted by sucrose and by dextrose are described. Mannose is a strong inhibitor of respiration. The inhibition is reversed by the simultaneous addition of those sugars which also reverse the growth inhibition caused by mannose. Mannose inhibits the respiration of sucrose and of glycolytic intermediates. Galactose is slowly respired and does not, even at high concentration, inhibit the respiration of sucrose.

Standards of Radioactivity

THE U.S. National Bureau of Standards Circular No. 594, entitled "Preparation, Maintenance, and Application of Standards of Radioactivity", by W. B. Mann and H. H. Seliger (pp. iv+47. Washington, D.C.: Government Printing Office, 1958. 35 cents), is a revision and unification of two review articles originally published in 1956 (*Internat. J. App. Rad. and Isotopes*, 1, 3 and 215; 1956). It surveys the current position in the field of standardization of radioactivity and describes the methods available for the preparation of both primary and secondary standards. The applications of the standards to problems in physics are discussed, including historical and geological dating from half-lives. One section is devoted to a summary of the results of the international comparisons of radioactivity standards which have been made during the past few years, and the circular concludes with an extensive bibliography. The main differences from the original articles are, apart from the inclusion of recent work, the use of a modified Harvard system of references and the incorporation of illustrations showing specifically the methods used at the National Bureau of Standards in the field of radioactivity.

Radio Industry Council Premiums for Technical Writing

THE Radio Industry Council has announced the award of six premiums of 25 guineas each for papers on radio and electronics published during 1958, as follows: "A New High-efficiency High-power Amplifier", in *The Marconi Review*, by V. J. Tyler (Marconi's Wireless Telegraph Co., Ltd.); "New Types of d.c. Amplifiers", Parts 1 and 2, in *Electronic and Radio Engineer*, by D. J. R. Martin (The Mining Research Establishment); "A Survey of Microwave Radio Communication", in *Electronic Engineering*, by W. J. Bray (Post Office Engineering Department); "All Travelling-wave Tube Systems", in *Electronic Engineering*, by S. Fedida (Marconi's Wireless Telegraph Co., Ltd.); "Electronic Developments at Very Low Temperatures", in *British Communications and Electronics*, by Dr. E. Mendoza (University of Manchester); "Analogue Computation", in *British Communications and Electronics*, by E. Lloyd Thomas (R. B. Pullin and Co., Ltd.).

Institution of Electronics Premiums

THE Institution of Electronics is to award the following new premiums for papers accepted for publication in the *Proceedings of the Institution of Electronics* during 1959: The Institution of Electronics Premium (25 gns.) for the most outstanding paper of the year; the Electronic Applications Premium (20 gns.) for the most outstanding paper dealing with the practical application(s) of electronics; the New Electronic Device Premium (15 gns.) for the most outstanding paper dealing with a specific new electronic device; the Institution of Electronics Exhibi-

tion and Convention Premium (20 gns.) for the most outstanding paper read at the 1959 Convention and accepted for publication in the *Proceedings*. Further particulars of the regulations governing these awards can be obtained from Mr. W. Birtwistle, Honorary General Secretary, Institution of Electronics, 78 Shaw Road, Rochdale, Lancs.

Carlsberg-Wellcome Travelling Research Fellowships

Applications are invited for the Carlsberg-Wellcome Travelling Research Fellowships, 1959–60, the object of which is to encourage co-operation, on an exchange basis, between Danish and British research workers in any branch of the natural sciences which has a bearing upon human and animal medicine. One fellowship annually is awarded to a candidate from the United Kingdom for a year's work in Denmark and one annually to a Danish candidate for a year's work in the United Kingdom. The stipend is in the range £800–£1,200 per annum (or the equivalent sums in Danish kroner), travelling and some incidental expenses being provided in addition. Tenure of the awards will start in September, 1959. Inquiries from candidates in the United Kingdom should be addressed to the Assistant Scientific Secretary of the Welcome Trust, 52, Queen Anne Street, London, W.1. Completed applications must be submitted before April 30.

Institute of Metals: Officers

The following were recently elected to office on the Council of the Institute of Metals: *President*, G. L. Bailey (director, British Non-Ferrous Metals Research Association); *Past-President*, Lord Tedder (chancellor, University of Cambridge); *Vice-Presidents*, Prof. H. Ford (Imperial College of Science and Technology, London); E. H. Jones (joint managing director, Capper Pass and Son, Ltd., North Ferriby); and Prof. H. O'Neill (University College of Swansea, University of Wales); *Honorary Treasurer*, D. P. C. Neave (director, British-American Metals Co., Ltd., Capper Pass and Son, Ltd., James Bridge Copper Works, Ltd., and Wolverhampton Metal Co., Ltd.).

Society for Analytical Chemistry: Officers

The following have been elected to office in the Society for Analytical Chemistry for the forthcoming year: *President*, R. C. Chirnside; *Past Presidents serving on the Council*, J. H. Hamence, D. W. Kent-Jones, J. R. Nicholls, K. A. Williams; *Vice-Presidents*, D. C. Garratt, Magnus A. Pyke, J. G. Sherratt; *Honorary Treasurer*, A. J. Amos; *Honorary Secretary*, R. E. Stuckey; *Honorary Assistant Secretaries*, L. Brealey (*Programmes Secretary*), S. A. Price.

University News: Belfast

The following appointments to lectureships in The Queen's University of Belfast have been announced: Dr. A. H. Jarrett (applied mathematics); Dr. R. W. J. McCarroll (applied mathematics, digital computing); Dr. M. W. Roberts (physical chemistry); Dr. S. C. R. Dennis (engineering mathematics); A. M. Arthurs (engineering mathematics); B. G. Leary (electrical engineering).

Bristol

A grant of £2,075 from the Department of Scientific and Industrial Research to the Department of Physics for research in cosmic rays under Prof. C. F. Powell for the year ending July 31, 1960, has been received. The following have been promoted to senior lectureships from August 1, 1959: D. F. Gibbs (physics); Dr. R. J. Fitzpatrick (veterinary pharmacology).

Leeds

The following appointments were recently made in the University of Leeds: Dr. A. Ralston, as temporary lecturer in the Department of Mathematics (Electronic Computing Laboratory); Dr. W. Rigby, as lecturer in the Department of Organic Chemistry; Dr. F. H. J. Cornish, as lecturer in applied mathematics.

Sheffield

The following appointments were recently made in the University of Sheffield: Mr. C. L. Kirk, to be lecturer in the Postgraduate Department of Applied Mechanics; and Mr. M. Cable, to be lecturer in glass technology.

Announcements

Dr. E. J. Solvay, of Brussels, has been appointed president of the Society of Chemical Industry in succession to Sir Robert Robinson, who will complete his term of office on July 10. Dr. Solvay is a direct descendant of the discoverer of the ammonia soda process which bears his name.

The Langley Memorial Prize was founded by the friends of the late Dr. W. H. Langley, principal medical officer, Southern Nigeria, to commemorate his work and the services he rendered to Nigeria. The prize, of approximately £21, is open to officers, past and present, of the West African Medical Departments; it is awarded periodically for the best paper on one of the following subjects: (*a*) tropical medicine or surgery; (*b*) tropical hygiene and sanitation; (*c*) tropical entomology and parasitology. Entries for the award in 1959, consisting of either published or unpublished work, should be sent to the Dean, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1, not later than October 1.

April 22 is the twenty-first anniversary of the foundation of the British Coal Utilization Research Association. To mark the occasion the Association is throwing its laboratories open to inspection during April 22–24. On April 22, which is to be a private members' day, the proceedings will be opened by the Rt. Hon. Lord Mills, Minister of Power. Further information can be obtained from the Assistant to the Director-General, B.C.U.R.A., Randalls Road, Leatherhead, Surrey.

The Clay Minerals Group of the Mineralogical Society is holding a meeting on Clay Minerals and Industry in Sheffield on April 15 and 16. Further information can be obtained from Dr. R. M. S. Perrin, School of Agriculture, The University, Cambridge.

The X-ray Analysis Group of the Institute of Physics is holding a conference on Clay Minerals and Biological Fibres in the School of Chemistry, University of Leeds, on April 17 and 18. Further information can be obtained from the honorary secretary of the Group, Dr. R. C. Gordon, Safety in Mines Research Establishment, Portobello Street, Sheffield 1.

THE CARNEGIE INSTITUTION OF WASHINGTON

REPORT FOR 1957–58

YEAR BOOK 57 of the Carnegie Institution of Washington* includes the President's and departmental and administrative reports for the year July 1, 1957–June 30, 1958, together with a bibliography of publications. Reviewing the work of the Institution, the president, Caryl P. Haskins, recalls that the year marked the tenth anniversary of the dedication of the 200-in. Hale telescope on Mount Palomar and of the joint operation of the Mount Wilson and Palomar Observatories by the Carnegie Institution of Washington and the California Institute of Technology. Both the presidential and the departmental reports briefly survey the work of the decade and the important advances in knowledge, particularly in our ideas of the size of the observable universe, the magnitudes of the distances within it, and its age, and in our knowledge of stellar evolution.

During the year the magnetic classification and study of sunspots and related phenomena continued, and a series of direct photographs of the bright comet Mrkos (1957*d*) was obtained with the 48-in. Schmidt telescope. An extensive project on the chemical composition of stellar atmospheres is in progress and a survey of high-dispersion spectra of the early carbon stars has revealed differences in both the line and the band spectra. Coudé spectrograms with a resolution of 10 A./mm., or better, are being accumulated to obtain evidence bearing on mass loss from late-type giants and super-giant stars, and the investigation of the internal motion of nebulæ using the multislit technique and a dispersion of 4·5 A./mm. at the 200-in. coudé continued. Work on the colour-magnitude diagram of the Draco system showed that the diagram is of the same type as that of the globular clusters of the galactic halo and conforms to the rule that large numbers of cluster-type variables can only be expected if the horizontal branch on the red side of the variable gap is strongly populated. The direct photography of multiple galaxies continued, and also the investigation of objects which might be associated with radio sources. A theoretical investigation was conducted by the properties of the very luminous *OB* stars observed by Code and Houck, and a preliminary model of a photoelectric spectrum-scanning device was tried on the coudé spectrograph of the 100-in. telescope. The Committee on Image Tubes for Telescopes has continued to encourage the development of photoelectric image-intensifying devices for use in astronomy, directing attention both to methods that record the photoelectrons themselves and to methods that use photoelectrons to produce an optical effect.

The Department of Terrestrial Magnetism continued its study of the emission of energy at radio frequencies from celestial objects, but solar activity of unprecedented magnitude interfered seriously with optical red-shift measurements of the far-distant galaxies at Mount Wilson and Palomar, although work proceeded rapidly on precision equipment designed to measure the position of radio sources to within a few square minutes of arc. The density distribution and velocity distribution of atomic hydrogen gas clouds in our galaxy continued to be of major interest to the radio astronomy group, while three noteworthy projects were carried forward as part of the programme of the International Geophysical Year: the study of the intense band of electric current, called the electro jet, that circulates in the upper atmosphere in the region of the Earth's magnetic equator; a study of the Earth's crust in the Andean highlands at more than 200 sites, utilizing explosions normally set off in operating large open-pit copper mines; and the measurement of the ages of rock minerals through the methods provided by the study of radioisotopes, notably the geographical patterns of the ages of Precambrian rocks in North America, particularly the metamorphic Precambrian rocks of the central and southern Appalachians. A general picture of the geological history of the Appalachian orogenic belt has emerged.

The Geophysical Laboratory has constructed new equipment of rather revolutionary design for studying geochemical and geophysical phenomena: with a new 'single-stage' apparatus, pressures of up to 50,000 atmospheres at temperatures up to 1,700° C. have been achieved, and in the two-stage design, in which the piston of the single-stage apparatus is supported, up to 65,000 atmospheres at 1,100° C. The single-stage apparatus is being used to study the influence of pressure on the melting-points of minerals that might exist 80 miles below the Earth's surface. Work on mineral assemblages in the Green River formation, on the beneficial effect of potassium on soils contaminated with strontium-90, on the effect of water on the melting of silicates and on the biotites is reported in some detail, as well as work on feldspar, ore minerals of the sulphide type and on the diffraction effects of short-range ordering in layered sequences. In crystallography the magnetic structure of chalcopyrite has been determined by neutron diffraction studies and a crystallographic study was made of arsenopyrite by X-ray diffraction methods.

Five of the Institution's seven departments are concerned with biological investigations of one kind or another. The Geophysical Laboratory, for example, has investigated the detection of organic materials in Precambrian rocks and has significantly increased the yields of organic materials from processed shales such as Swedish Kolm shale (Cambrian), Nevadan Vanini shale (Ordovician) and Colorado Green River shale (Eocene). In the Biophysics Group of the Department of Terrestrial Magnetism attention has been focused particularly on the critical importance in metabolism and growth of the elaborate and precise fine structure of many of the constituents of the living cell. The properties of the ribosomes and the kinetics of their formation have been studied by ion-exchange columns, radioactive-tracer methods and the 'Spinco' Model *L* centrifuge. Work with *Escherichia coli* is described in some detail, while,

* Carnegie Institution of Washington. Year Book No. 57, July 1, 1957–June 30, 1958. Pp. xl+497+12 plates. (Washington, D.C.: Carnegie Institution, 1958. 1 dollar, paper bound; 1.50 dollars, cloth bound.

besides studies on virus purification, extensive work on amino-acid analogues continued with particular emphasis on determining whether the analogues are contained in radically different molecular species or in proteins similar to those normally synthesized.

Measurements in the Department of Plant Biology on the derivative absorption spectra of chlorophyll in numerous algae and other plants suggest that there are more than two forms of chlorophyll *a*. Further investigation on the photochemical formation of chlorophyll in leaves from its precursor protochlorophyll gave yields of about 0·5–0·7 molecule of chlorophyll formed per quantum of light absorbed, suggesting that two quanta of light may produce one molecule of chlorophyll. A study of the reversible oxidative bleaching of chlorophyll by chemical treatment and by light in several species of photosynthetic bacteria showed that the chemical properties of the pigment are markedly influenced by its incorporation in natural structures, the form absorbing light at the longest wave-length (890 mμ) being the most active. A number of climatic races of *Mimulus* plants are being grown for laboratory studies of the way their rates of respiration respond to variations of temperature and intensity of light, and studies have been commenced on the germination of seedling populations of contrasting parental and hybrid lines of *Mimulus* under crossed gradients of controlled temperature and light intensity.

In the Department of Embryology, studies of the molecular basis of rhythmic motility in spermatozoa indicated that the mechanisms of contractility and co-ordination can be disengaged experimentally. Important findings are reported in work on the development of the adrenals and sympathetic paraganglia in insectivores, and studies on the synthesis of the muscle proteins actomyosin and myosin in the regenerating limb of the salamander continued. Other work was concerned with differentiation and morphogenesis in the human embryo, the neural crest and its derivatives, the chemical basis of morphogenetic movements, the acquisition of biological specificity, the effect of hormones in developmental systems, and the anatomy and physiology of the uterus and the placenta.

In the Department of Genetics, studies with maize clarified an obscure aspect of the suppressor–mutator system of control of gene action, and analyses of biological ultrastructure by methods combining cytochemistry and electron microscopy offered new evidence that the chromosomes in actively dividing cells contain ribonucleic acids at all stages of the mitotic cycle. Studies of the intracellular deoxyribonucleases were concentrated on ascertaining the best procedures for extracting and purifying the intracellular deoxyribonuclease of salmon testes. Further studies of the properties of phage *T2*, which grows on strain *B* of *Escherichia coli*, suggest that phage particles are made from at least two protein sub-units, each containing a large fraction of the total phage sulphur. Studies of bacterial genetics with *Salmonella typhimurium* and *Escherichia coli* support the assumption that recombination among five genetic markers in the former is accomplished through a 'copy-choice' mechanism governing the exchange of characters between a recipient chromosome and a transducing element, and that the frequency of 'switchovers' in this process depends on the genetic constitution of the chromosome regions. Evidence has been obtained that transduction by bacteriophage lambda of loci concerned with galactose utilization in *E. coli* is a matter of lysogenization by a new type of genetic structure, which is part phage and part bacterial in origin.

The Department of Archæology completed its work during the year, and both the departmental and the presidential reports review broadly its work over more than fifty years. Some notes on ceramic studies in Yucatan and a ceramic technology are included in the departmental report.

THE NATIONAL RESEARCH DEVELOPMENT CORPORATION

REPORT FOR 1957–58

THE report of the National Research Development Corporation for the year ended June 30, 1958*, records a revenue from inventions of £174,242, notably from triiodothyronine, Prof. R. L. Wain's phenoxybutyric acid weed-killers, the Bailey bridge, the Royal Aircraft Establishment fire and heat detector and gyroscopic control apparatus for aircraft, the Ministry of Supply electrical ignition system and exploder, anti-thyroid compounds, the Hutchinson–Scarrott pulse height analyser, hecogenin from sisal, the Denny–Brown ship stabilizer control gear, nisin and anti-tumour agents of the nitrogen mustard type, on which all significant expenditure has ceased. Further reference is made to the Corporation's part in the development of electronic digital computers, including a project for the development of a large business-type computer and data-processing system of advanced design and performance, making extensive use of transistors, and two projects for magnetic type equipment for use with digital computers, but progress with the development of a very high-speed computer has been slow. An agreed programme of research and development of computer-controlled machine tools was initiated at the Manchester College of Science and Technology in January 1956, and interesting results are being obtained on the performance of slideways and on the cutting forces occurring during contour milling operations. Work continues on the development of high-performance drive systems, but the Corporation now considers that the time has come to withdraw its financial support.

Work at the National Physical Laboratory on Sir Thomas Merton's diffraction grating inventions is proving important in unexpected ways, and the Corporation's intervention in the development of printed electrical circuits has also been fully justified by results. The Corporation has now been able to retire from the collaborative effort it initiated seven years ago, leading to the collection of pituitary gland and production of adrenocorticotropic hormone on a commercial basis. Development of a process for

* National Research Development Corporation. Report and Statement of Accounts for the year 1st July, 1957, to 30th June, 1958. Pp. ii+28. (London: H.M. Stationery Office, 1958.) 1s. 6d. net.

producing aldosterone in significantly greater quantities from adrenal glands, including a limited amount of aldosterone and other adrenal hormones labelled with tritium, is also being financed, as well as a project for the isolation from *Pueraria mirifica*, from Thailand, of the œstrogenic substance, 'mircestrol', and elucidation of its structure, with the view of ultimate synthesis and medical use.

The four prototype groundnut harvesters shipped to East Africa in the spring of 1958 operated well on the true bunch varieties for which they were designed, but some difficulties were encountered in handling semi-bunch varieties, and commercial applications have been established for flexible barges of 40 to 1,000 tons capacity for transporting oil and other liquids by water. Among the new projects, some of which the Corporation proposes to attack under the extended powers confirmed by the 1954 Act, are a cotton-spinning project at the British Cotton Industry Research Association; a continuous-flow laundering project at the British Launderers' Research Association; a programme on ferroelectric ceramics at the Battersea College of Technology; the development of Prof. J. McGee's new form of image intensifier at the Imperial College of Science and Technology; the National Institute of Agricultural Engineering's jute ribboner; and an ultrasonic flow-meter at the British Scientific Instrument Research Association.

Government departments and research councils assigned patent rights in 86 cases compared with 105 in 1956–57, 49 of the total of 147 coming from the universities and eight from private firms and individuals in the United Kingdom, compared with 26 and 3, respectively, out of 148 in 1956–57. Of 612 inventions communicated to the Corporation during the year, compared with 631 in 1956–57, 231 were from Government departments and research councils, 13 from official organizations, four from charitable organizations and 286 from private firms and individuals, 265 in the United Kingdom. Holdings of British and foreign patent applications totalled 2,930, including 370 United Kingdom applications and 595 granted patents, 975 overseas applications and 990 granted patents.

FIGURAL AFTER-EFFECTS

PETER McEWEN'S monograph supplement to the *British Journal of Psychology*, entitled "Figural After-effects" (Monograph Supplement 31; 1958), is a timely summary of research and theorizing in a rapidly growing field of experimental psychology. The fundamental facts are relatively simple and universally accepted; they were first observed by the American psychologist, J. J. Gibson, who found that subjects who had inspected a curved line for some time would afterwards see a straight line as curved in the opposite direction. This discovery aroused relatively little interest until it was taken up by one of the great figures of psychology, Wolfgang Köhler, who had been one of the founders of the *Gestalt* school. He showed in a wealth of detail that almost any kind of visual or tactual perception produced certain effects of a relatively lasting character, effects which would change subsequent perceptions and which could be measured by the amount of such change induced. Most of his work was concerned with visual stimuli, but he also initiated experiments in kinæsthetic figural after-effect, and other sense modalities have also been studied since. It was Köhler who coined the term 'figural after-effects' by which these phenomena are now known.

Köhler did more than this, however. He proposed a physiological theory of the origin of these phenomena, making use of the concept of electrotonus, and deducing the observed phenomena from this theory. In recent years this has led him right over into the physiological field, where his vigorous and unorthodox approach has caused a good deal of discussion and criticism. Within the psychological field, however, he did one further great service by pointing out that his general theory could serve to explain many previously unrelated phenomena, such as the disappearance of certain visual illusions with time, and by suggesting that there were certain very striking similarities between the satiation process, which he believed to underlie figural after-effects, and certain inhibitory phenomena well known in the field of learning and conditioning. Köhler's theory therefore holds out an exciting prospect of unification of large areas of psychology which hitherto had been treated as quite separate from each other; this fact alone would justify the great amount of work that has gone into the testing of his hypotheses.

Dr. McEwen traces all this work, both on the experimental and theoretical level, in a painstaking, accurate and detailed account which is always judicious and remains close to the facts. He shows how much of the work that has been reported suffers from being purely phenomenological rather than quantitative, and how this lack of quantification has made any decisive type of experimentation or theorizing extremely difficult. To take but one example, after-effects are sometimes said to disappear after a few minutes, while at other times they are said to last for days or even months. McEwen also shows very clearly how complex the phenomena under consideration are, and how many of them require *ad hoc* hypotheses to be brought under the wing of Köhler's theory—or indeed that of the only rival theory, advanced by Osgood and Heyer.

It would probably be true to say that concentration on *apparently* unimportant issues and laboratory investigations of problems of theoretical interest characterize a maturing science, whereas exclusive preoccupation with direct practical questions is often characteristic of a pre-scientific stage. Similarly, the emergence of broad general theories covering a large amount of ground, and unifying many diverse phenomena, is also characteristic of a stage of relative maturity in a science, as compared with the purely empirical *ad hoc* investigation of unrelated phenomena. Learning theory was the first part of psychology to achieve such a level of maturity, and Köhler's work may now be said to have achieved the same status for perception. Indeed, the fact that he was able to point to very striking similarities between phenomena in perception and in the learning field suggests that an even greater unification may be in prospect. The whole situation is an exciting and promising one, and inevitably reminds one of those halcyon days in atomic research when interest was

still confined to Bohr's model and Heisenberg's principle. The debt which psychology owes to Köhler had already been a large one before he investigated figural after-effects; his work in this field has almost certainly made him one of the greatest figures in psychology. It is curious and a little piquant to realize that the concepts which Köhler uses to account for figural after-effects are similar in many ways to those used by Pavlov to account for the facts of conditioning. That this should be so, in spite of the fact that not so very long ago Köhler and his colleagues founded the *Gestalt* school as an express antithesis to Pavlov's 'atomistic' and associationistic theorizing, illustrates the relative unimportance of the critical parts of the contribution made by schools in psychology, and the much greater importance of their positive and experimental contributions. We have fortunately now left behind to a large extent this era of rivalling schools, and it is fortunate that Köhler survived into and indeed partly originated this new period. The reader interested in the history of science can do no better than read through Dr. McEwen's monograph to discover both the promise of the present situation and also the severe birth-pains to which it is giving rise.

H. J. Eysenck

IRON ORE IN THE SOVIET UNION

"THE iron ore deposits of the Kursk magnetic anomalies, both as regards their concentration and their quality, have no rivals in the world"—so writes P. Y. Antropov, the Minister of Geology in the U.S.S.R. (*Priroda*, 7, 16; 1958). Magnetic anomalies were first discovered during a topographic survey in the region of Kursk in 1783. Before the 1917 revolution, E. E. Leist, professor in the University of Moscow, made a careful survey of magnetic anomalies and concluded that they are due to the presence of magnetite ores at great depths below the cover of sedimentary rocks. The results of the 1919–26 survey were rather disappointing; only rather poor iron ores were found and at a considerable depth. Renewed explorations during 1930–34 and the construction of an experimental shaft (flooded and ruined in 1938) revealed rich ore, but again at a great depth and in a very difficult geological setting. Exploration, on a much wider scale, was resumed after the Second World War. This detailed survey revealed two important features of the geological structure of this region:

(1) Below a cover, varying in thickness from 40 to 500 m. or more, the pre-Palæozoic floor revealed a gigantic anticlinal fold, or rather a packet of such folds forming a belt about 150 km. × 250 km. in extent stretching in a north-west–south-east direction, with the line Orel–Kursk–Belgorod as its diagonal. This pre-Palæozoic massif was buried under a cover of Palæozoic, Mesozoic and Tertiary sedimentary rocks, and was made of Proterozoic schists, ferruginous quartzites and gneisses.

(2) The rich iron ore was not found among the Proterozoic ferruginous quartzites but in a secondary iron ore formed as a crust of weathering immediately above the Proterozoic floor. This secondary iron ore was formed through an almost complete leaching out of silica from the ferruginous quartzites and a transformation of magnetite into martite (hæmatite pseudomorphs after magnetite). The maximum thickness of this ore deposit occurs in the Belgorod district, where it reaches 350 m. Elsewhere, it varies from 30 to 50 m. The best-quality ore occurs in the Yakovlevsky district, where it contains on average iron 61·4 per cent, silicon 5 per cent, sulphur 0·1 per cent and phosphorus 0·02 per cent.

The reserves of ores in the Belgorod region alone are 12 milliard tons, that is to say, five times the reserves of the Krivoy Rog iron ore deposit, until now the largest known in the U.S.S.R. The total reserves of the iron ores in the region of the 'Kursk magnetic anomalies' may be as high as 15–20 milliard tons.

The winning of these ores has already begun by means of shafts and open-cast quarries in the Lebedinsky, Mikhailovsky and Yakovlevsky districts. Thus in the Lebedinsky district the open-cast extraction amounted to 6 million tons in 1956; the Mikhailovsky district is planning to extract 6½ million tons per annum, and the Yakovlevsky district 15 million tons. Thus the total production of these three districts by 1966 is estimated at 27½ million tons per annum. These figures suggest that the total extraction of rich iron ore in the whole region may be estimated at 70–80 million tons per annum. This amount of ore would correspond to 30–35 million tons of cast iron per annum.

In this connexion it is important to note that in 1957 the total production of iron ore in the Soviet Union was 84·2 million tons and the total production of cast iron 37 million tons per annum.

S. I. Tomkeieff

EMPLOYMENT OF SCIENTISTS AND TECHNOLOGISTS IN THE UNITED STATES

ACCORDING to a preliminary report from the National Science Foundation, American industry in January 1957 employed 738,000 engineers and scientists (about two-thirds of all those in the United States), of whom 58,000 were employed as administrators of scientific and engineering activities. The largest occupational group was 528,000 engineers, chemists (72,000) coming next, followed by medical, agricultural and biological scientists (16,600), geologists and geophysicists (14,200), physicists (12,100), mathematicians (12,400) and metallurgists (10,800). Of the total, 92,900 were employed in the electrical equipment industries, 84,900 in the aircraft and parts industry, 79,200 in chemicals and allied products industries, 74,100 in machinery, 50,700 in petroleum products and extraction, 30,200 in fabricated metal products and ordnance, 29,500 in primary metals, and 24,600 in the professional and scientific instruments industry; 29,000 more chemists were employed in chemical industry than in any other industry.

Almost one-third (228,000) of these engineers and scientists were engaged in research and development, and two-thirds of these were employed in the aircraft, electrical equipment, machinery or chemical industries. Nearly three out of every five physicists, but fewer than one out of three of the engineers, mathematicians and biological scientists and two out of every five chemists were engaged in research and development. Nearly 600,000 technicians worked with scientists and engineers in all activities, and of these, 160,000 were engaged in research and development, the largest numbers in the electrical equipment, aircraft, machinery, chemical and fabricated metal parts and ordnance industries. During 1954–57 employment of engineers rose by 27 per cent, of chemists by 16 per cent and of biological scientists and physicists by about 60 per cent. In these three years 70,000 first degrees in engineering were awarded by American engineering; the proportion of engineers and scientists employed in research and development rose from 28 to 31 per cent, and employment in such activities rose by more than 45 per cent, whereas in the same period research and development costs rose from 3,700 million to 6,400 million dollars.

CHEMOTHERAPY OF PARASITIC DISEASES

A MEETING of the Parasitology Group of the Institute of Biology was held at the Institute's rooms in London on October 24, when two speakers were invited to read papers on the chemotherapy of parasitic diseases.

Dr. Ann Bishop (Molteno Institute, Cambridge) described some aspects of resistance to drugs in parasitic Protozoa. The fact that parasitic organisms can become resistant to drugs in the course of the therapeutic treatment of the host was discovered in Ehrlich's laboratory, early in the present century, by Franke and Röhl while studying the effect of parafuchsin upon *Trypanosoma brucei* in mice. Trypanosomes have since been made resistant to a wide variety of chemical compounds.

The fundamental problems in the study of resistance to drugs are the nature of the changes which organisms undergo in becoming resistant and the means by which these changes are brought about. In trypanosomes, resistance to drugs may be associated with changes in permeability and with changes in the enzymes present in the cells. Drug resistance in these organisms may be a very stable character and persists after the transmission of the parasite through the insect vector. The spontaneous development of resistance, in the absence of the drug, has been described both in trypanosomes and in *Toxoplasma gondii*.

Malaria parasites also can be made resistant to certain compounds, including proguanil and pyrimethamine, which are used in the treatment of the disease. Resistance persists after transmission of the parasite through the insect vector, in which, unlike trypanosomes, the malaria parasite undergoes a sexual cycle of development.

A study has been made of the development of resistance to metachloridine (2-metanilamido-5-chloropyrimidine) in pure lines of *Plasmodium gallinaceum* in chicks. No difference in the rate of development of resistance was observed in these lines. The rate of development of resistance was not related to the size of the dose of drug, but was affected by the size of the inocula producing the infections exposed to the action of the drug. The suddenness with which resistance appeared in some of the strains studied, and its stability in the absence of the drug and after the sexual development of the parasite, suggested that it arose by mutation, the mutation being of a low frequency.

Dr. O. D. Standen (Wellcome Laboratories of Tropical Medicine, London) outlined an approach to the experimental chemotherapy of helminthiases. The diseases of man and animals caused by the presence of helminth parasites are particularly important in the tropics, and are often responsible for the low level of efficiency and uneconomic production of stock animals in rural areas. The eventual control of these helminthiases will depend on a fuller knowledge of their epidemiology, but also presents problems which can only be resolved by the use of drugs.

The number of even moderately efficient anthelmintics in use at the present time is small, and most are toxic to the host. There is thus an urgent need for drugs which could be used in mass treatments of man and animals.

The discovery of new drugs requires the close collaboration of biologist and chemist. Two methods of approach may be used. An empirical method is one whereby large numbers of compounds of unknown activity are tested, in order to determine their effects on the parasite. The selective approach is one in which chemicals of known anthelmintic potency are modified in order to improve their action, and this method may be used with substances discovered by the empirical method. The ultimate aim is to discover the relation between the molecular structure of the drug and its anthelmintic activity and toxicity to the host.

The selection of helminths to be cultured in laboratory animals is of great importance. The number which can be cultured in this way is small, and of these the species pathogenic to man and animals are few. It is thus necessary to test drugs using helminths which are the natural parasites of laboratory animals. The successful maintenance of a parasite in all the stages of its life-history in laboratory animals often requires a long period of fundamental research before a sufficient supply of material is assured for routine investigations, for which an abundance of material is required since hundreds or even thousands of tests may be necessary before an effective drug is discovered.

The use of a multiple helminth screen, employing a wide range of species living in different habitats, yields interesting results. Thus, basic work with *Aspicularis tetraptera* in the mouse has contributed materially to the development of piperazine for the treatment of ascariasis and oxyuriasis. More recent chemotherapeutic tests, against *Nippostrongylus muris* in the young rat, provided the basis for the development of salts of bephenium that are efficient in the treatment of nematodiriasis in sheep and ancylostomiasis in man and dogs.

The experimental chemotherapy of helminthiases necessarily demands many species of helminths in all

stages of their development and this offers an unparalleled opportunity for concurrent research into the structure and life-cycles of the parasites, into their biochemistry and host–parasite relationships and into the mode of drug action. While such studies may be an end in themselves, they also provide the only basis for a rational approach to chemotherapy.

In opening the discussion, Dr. H. O. J. Collier (Department of Pharmacological Research, Parke, Davis and Co., Ltd., Hounslow) said that the history of practical chemotherapy began with the treatment of parasitic diseases. Experimental chemotherapy also started in this field. In 1891 Ehrlich and Guttmann (*Berl. Klin. Wsch.*, **28** (9), 953; 1891) showed that methylene blue is a fairly potent antimalarial agent. Choosing this dye because it stained the malaria parasite within red corpuscles and because it was of low toxicity to man, Ehrlich, with Guttman's help, treated with it two sufferers from malaria and achieved immediate success. Later, Ehrlich had to test hundreds of compounds to achieve '606' and did not screen at random but approached his objective by steps, basing each new compound on results obtained with previous ones.

Work described in the preceding papers follows on the experimental chemotherapy started by Ehrlich. Each speaker was mainly concerned with one major problem, well known to Ehrlich and his colleagues; Dr. Bishop with resistance to drugs, and Dr. Standen with specificity of interaction between chemical and biological species.

Some of the ways in which drug resistance can develop were described. Compared with a sensitive organism, a resistant form may absorb a drug less, excrete it faster, destroy it more effectively, produce more of the drug's antagonist or possess a route of biosynthesis alternative to that blocked by the drug. These differences may depend on modifications of enzyme systems; an understanding of these would lead to a better understanding of drug resistance.

Problems of specificity are at the heart of chemotherapy, because if drugs were not more toxic to one species than to another, selective poisoning would be impossible. The differences in responses of various species to the same compound provide difficulties, however, because related species cannot be relied upon to respond in the same way to a new substance, and the second paper explained how the experimental chemotherapist deals with this dilemma.

Another aspect of the use of chemotherapeutic agents was mentioned by Dr. S. Kendall, in reference to coccidiosis of fowls. Drugs are added to feed in order to determine whether they are toxic to the parasite or whether resistance is developed. Results, so far, show these drugs to have a coccidiostatic action, without elimination of the parasite. Dr. L. Joyner said that this posed the general problem as to whether eradication of the parasite was necessary; in the case of flukes, for example, it was desirable. However, in some protozoan infections, it is preferable to induce a state of premunition in the host, or one of immunity induced by stages in the life-cycle other than the pathogenic one. Dr. J. Leiper said that some anthelmintics effective over a wide range may produce curious results, some parasites being killed and others encouraged. Thus, in the use of chemotherapeutic agents the habitat of the parasite within the host may be as important as the specific action of the drug. Dr. Standen replied that the range of activity of the drug must be carefully tested. Dr. C. Hopkins pointed out that the mode of action of the drug is unknown, a situation complicated by the difficulties of cultivation of parasites *in vitro*. Anthelmintics can act by blocking the metabolism of the worm, not necessarily causing a complete 'knock-out' reaction, and the site of the parasite within the host might indicate specific substances required from the host by the metabolism of the parasite.

The proceedings of this meeting will not be published in full.

June Mahon

A PRESUMPTIVE HUMAN *XXY/XX* MOSAIC

By Dr. C. E. Ford

Medical Research Council Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Berks

AND

Dr. P. E. Polani, J. H. Briggs and Dr. P. M. F. Bishop

Guy's Hospital, London Bridge, S.E.1

THE discovery of 'sex chromatin' by Barr and Bertram[1] and the subsequent recognition of its regular occurrence in normal females of many mammalian species (including man) has provided a new approach to the study of human intersexual conditions, the value of which is demonstrated in the proceedings of a recent symposium[2]. Although the presence of sex chromatin is generally taken to be diagnostic of an *XX* individual, this is not proved, and the unequivocal determination of the sex chromosome constitution can only come from the direct study of the chromosomes themselves.

A technique for obtaining chromosome preparations from biopsy specimens of bone marrow has recently been described in outline and used in the study of one 'chromatin-positive' case of Klinefelter's syndrome[3]. (Klinefelter's syndrome is a variable clinical syndrome in somatically male individuals who are sterile, generally with azoospermia, often with gynæcomastia and endocrine defects, but essentially and always with small testes after puberty.) The few cells from this first case seen in metaphase contained chromosomes indistinguishable from those of a normal female. Recently we have been able to examine the chromosomes from two further cases of this syndrome. One, chromatin-negative, had the chromosomes of a normal male. The second, chromatin-positive, gave an unexpected result and is the subject of this article.

A marrow specimen was obtained by sternal puncture at Guy's Hospital, converted into a suspension there and immediately sent to Harwell for

cytological processing. The final preparations contained a satisfactory number of cells in mitotic metaphase, although many of them were unsuitable for accurate observation owing to supercontraction of the chromosomes, or separation of the chromatids at the centromeres, or both. Sixty-five cells were accepted as suitable for counting, and the results are summarized in Table 1. In ten cells, including those recorded as containing 48 and 49 chromosomes, there was an uncertainty of one, or two, chromosomes. The method of counting (to minimize subjective bias) and the errors to which the recorded counts are liable have been discussed previously[3,4].

Table 1. COUNTS OF CHROMOSOMES IN 65 CELLS. KLINEFELTER'S SYNDROME (CHROMATIN-POSITIVE)

No. of chromosomes	41	42	43	44	45	46	47	48	49
No. of cells	1	0	0	2	3	13	44	1	1

We have not yet succeeded in identifying the individual *X* and *Y* chromosomes, although it has been reported that *Y* is distinguishable from other similar chromosomes in preparations made from cells in tissue culture[5]. Nevertheless, it is possible to identify sex cytologically by classifying the chromosomes into four clearly defined groups[3]: (1) the shortest chromosomes have nearly terminal centromeres; there are four in the female, five in the male; (2) the next longer ones form a mixed group; there are sixteen in both sexes; (3) the medium-length chromosomes have sub-median or sub-terminal centromeres; there are sixteen in the female, fifteen in the male; (4) the longest ten chromosomes are well differentiated and form five easily recognized pairs that are the same in both sexes. It follows that *Y* is a short chromosome with a nearly terminal centromere and that *X* is a medium-length chromosome with a sub-median or sub-terminal centromere. This is in accord with observations on the sex chromosomes bivalent at metaphase in first spermatocytes[6].

Twelve of the cells with 47 chromosomes from the present case were selected for detailed analysis. All were found to contain five very short acrocentric chromosomes plus sixteen other short chromosomes (as in a male) and sixteen chromosomes in the medium-length group (as in a female). The remaining ten chromosomes were the five long pairs common to both sexes. An example is shown in Fig. 1. The obvious interpretation is that these cells contain two *X*-chromosomes and one *Y*-chromosome, in addition to the normal 22 pairs of autosomes, although the possibility that they are basically female and trisomic for one of the small acrocentric autosomes is not excluded. In so far as the 47-chromosome cells are concerned, our case is therefore parallel to that described very recently by Jacobs and Strong[7].

An attempt was made to classify the chromosomes in all thirteen cells with counts of 46. This was possible in eleven cells, although not all were of equal technical standard. Five of them contained an apparently normal female set with *four* small acrocentrics, sixteen other short chromosomes, sixteen medium-length and ten long chromosomes. There were points of uncertainty in the remaining *six cells, but at least three of them probably had the* same constitution. We therefore believe the case to be an instance of true mosaicism, notwithstanding the likelihood that some counts of 46 may represent damaged cells that originally contained 47 chromosomes. *In the first place, there are many more cells* with a count of 46 than would be expected (in our experience) from damage alone; and secondly, the chance of loss of one of the same group of five chromosomes from at least five, probably eight, and possibly more of the eleven cells must be very low indeed.

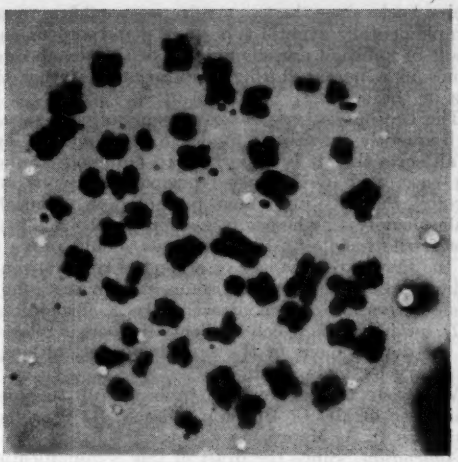


Fig. 1. Cell in metaphase with 47 chromosomes (presumptively *XXY* plus 44 autosomes) from the bone marrow of the patient with Klinefelter's syndrome (chromatin-positive). Feulgen squash after colchicine pretreatment. (× 2,220)

Granted that the 47-chromosome cells are *XXY* and the case is a true mosaic, it is most reasonable to suppose that the 46-chromosome cells arose through loss of the *Y*-chromosome by mitotic non-disjunction. It is known that clones of reticular cells, characterized by one or more visible chromosome rearrangements, may appear during the regeneration of the bone marrow in irradiated mice[8,9]; and recent evidence has shown that in certain circumstances they may proliferate differentially even to the extent that one clone is disseminated widely and outgrows all the rest (Ford, C. E., Micklem, S., and Gray, S. M., unpublished work). There is also evidence that marrow cells deficient in chromosome segments produced by irradiation of normal animals are very rapidly eliminated (Sharman, G. B., unpublished work). Now, loss of the *Y*-chromosome from an *XXY* cell would be a step in the direction of *normality*, and such cells might therefore exhibit a small selective advantage relative to their progenitors; on the other hand, loss of an autosome would yield a deficient cell, which, if it did not die, would not be expected to multiply. We therefore assume that the 46-chromosome cells contain two *X*-chromosomes and no *Y*-chromosome, that is, contain the normal female complement.

The supposition is that the mosaic patient developed from an *XXY* zygote and that this arose from fertilization of a non-disjunctional *XX* egg by a normal *Y* sperm, or of a normal *X* egg by a non-disjunctional *XY* sperm. The occurrence of one non-disjunctional type immediately suggests that others may be found. These could include *XXX*, *X0*, *Y0*, *and possibly XYY types; some* of them have indeed been postulated to exist on various grounds. Of these, perhaps, the most likely candidate is *X0* for chromatin-negative cases of Turner's syndrome[10]. The occurrence of an individual with Klinefelter's syndrome and one with Turner's syndrome in the same sibship[11] is readily explained on

such a basis, since the two syndromes would represent complementary zygotic types and could appear as a result of habitual non-disjunction of the sex chromosomes in a parent during maturation of either the ova or the sperm.

The *Drosophila* type of sex determination has been so frequently taken as a model in texts and reviews of all sorts that it is important to point out that in our case, and in the case reported by Jacobs and Strong[7], the *XXY* individual presented an essentially male phenotype, whereas in *Drosophila* the *XXY* fly is a fertile female[12]. If these cases should prove to be the first two instances of a general rule, it would indicate that the *Y*-chromosome in man is strongly 'male-determining', in contrast to the *Y*-chromosome of *D. melanogaster*, which is essentially inert in relation to the development of the sex phenotype, although necessary for male fertility[11].

We wish to acknowledge the skilful technical assistance of Mr. G. Breckon, Miss S. R. Wakefield and Miss P. A. Moore.

Addendum. Since the draft of this communication was completed, we have, in conjunction with our colleagues, Dr. D. G. Harnden and Dr. K. W. Jones, examined the chromosomes of three more chromatin-positive cases of Klinefelter's syndrome and of one chromatin-negative case of Turner's syndrome. The former all have 47 chromosomes, show no evidence of mosaicism and are presumptively *XXY* individuals. Forty-five chromosomes were found regularly in bone marrow cells from the patient with Turner's syndrome, and analysis of the detailed chromosome morphology is consistent with the anticipated X0 constitution. (Miss Jacobs and Dr. J. S. S. Stewart have kindly allowed us to state that they have found 45 chromosomes in a chromatin-negative case of Turner's syndrome, and that their observations are also consistent with X0 interpretation.)

[1] Barr, M. L., and Bertram, E. G., *Nature*, **163**, 676 (1949).
[2] Symposium on Nuclear Sex, edit. D. Robertson Smith and W. M. Davidson (Heinemann, London, 1958).
[3] Ford, C. E., Jacobs, P. A., and Lajtha, L. G., *Nature*, **181**, 1565 (1958).
[4] Ford, C. E., Hamerton, J. L., and Mole, R. H., *J. Cell. Comp. Physiol.*, **51**, Supp. 1, 235 (1958).
[5] Tjio, J. H., and Puck, T. T., *J. Exp. Med.*, **108**, 259 (1958).
[6] Ford, C. E., and Hamerton, J. L., *Nature*, **178**, 1020 (1956).
[7] Jacobs, P. A., and Strong, J. A., *Nature*, **183**, 302 (1959).
[8] Ford, C. E., Ilbery, P., and Loutit, J. F., *J. Cell. Comp. Physiol.*, **50**, Supp. 1, 109 (1957).
[9] Barnes, D. W. H., Ford, C. E., Gray, S. M., and Loutit, J. F., Proc. *Second Int. Conf. Peaceful Uses of Atomic Energy*, Geneva, 1958. Vol. 6, Biological Sciences (in the press).
[10] Polani, P. E., Lessof, M. H., and Bishop, P. M. F., *Lancet*, ii, 118 (1956). Danon, M., and Sachs, L., *ibid.*, ii, 20 (1957).
[11] Bassøe, H. H., *J. Clin. Endocrinol.*, **16**, 1614 (1956).
[12] Bridges, C. B., in "Sex and Internal Secretions", edit. E. Allen (*Baillière, Tindall and Cox, London*, 1939).

TRANSPHOSPHORYLATING ENZYMES AND MUSCLE RELAXATION

By J. MOLNAR and Dr. L. LORAND

Department of Chemistry, Northwestern University, Evanston, Illinois

THE pure creatine phosphate or the phosphoenolpyruvate kinase system greatly enhances the reversible relaxation of glycerinated rabbit muscle[1] (fibre bundles or single fibres) after contraction with adenosine triphosphate[2–5]. Transphosphorylation (presumably to adenosine diphosphate), though essential, is by itself insufficient to cause relaxation of old or specially extracted fibres. In such cases, in addition, the presence of a microsomal (or granular) fraction from muscle homogenate is required for relaxation. This has been demonstrated for the isotonic condition with creatine phosphate[6–8], and for isometric relaxation in conjunction with phosphoenolpyruvate[4,5].

Although the role of the microsomal principle for relaxation is generally accepted, the function of the transphosphorylating enzymes is regarded by many as 'unphysiological'[9–13]. It is claimed that the need for transphosphorylation arises only if the penetration by diffusion of adenosine triphosphate into the fibres cannot match the hydrolysis of the nucleotide by the myofibrillar adenosine triphosphatase. The addition of an enzyme system which restores adenosine triphosphate would merely ensure that the interior of the fibre maintains the necessary level of adenosine triphosphate similar to that prevailing in the bath in which the fibre is immersed. These contentions are based mainly on the work of Weber and his collaborators, and that of Bendall, who could not demonstrate any need for the addition of transphosphorylase in Marsh's system[14] in which, instead of fibres, homogenized myofibrils of microscopic dimensions are used. The experiment can be per-

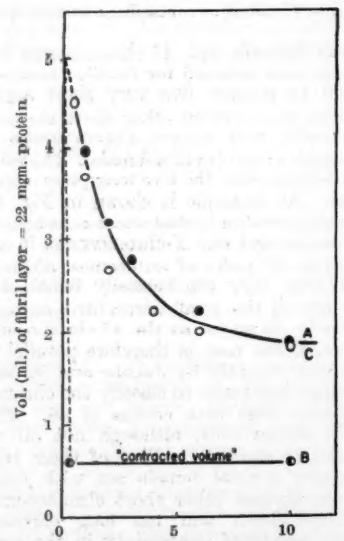


Fig. 1. Sedimentation of myofibrils. Magnesium chloride, 4 m*M*; pH about 6·5; $\mu \approx 0{\cdot}15$; total vol., 5 ml. *A*, fibrils (about 22 protein-mgm.—ref. 15) alone; *B*, fibrils + (1·04 protein-mgm. microsomes + 2·6 m*M* phosphoenolpyruvate —ref. 16) or (microsomes + 180 μgm. phosphoenolpyruvate kinase—ref. 17) + 4 m*M* adenosine triphosphate; *C*, fibrils + microsomes + phosphoenolpyruvate + phosphoenolpyruvate kinase + adenosine triphosphate

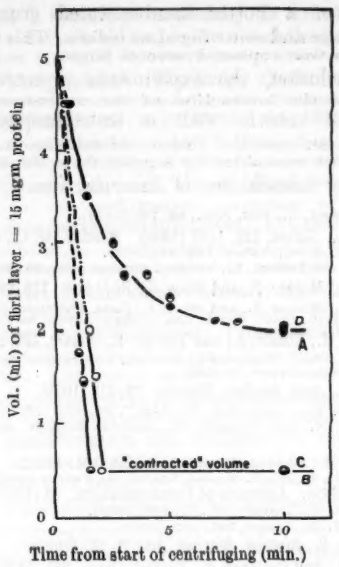


Fig. 2. Sedimentation of myofibrils. Conditions same as in Fig. 1. *A*, fibrils (about 15 protein-mgm.) alone; *B*, fibrils + 4 m*M* phosphocreatine (ref. 18) + 20 μgm. creatine phosphate transphosphorylase (ref. 19) + 4 m*M* adenosine triphosphate; *C*, fibrils + 1·52 protein-mgm. microsomes + adenosine triphosphate; *D*, fibrils + microsomes + phosphocreatine + creatine phosphate transphosphorylase + adenosine triphosphate

formed either by observing the packing volume of the myofibrillar layer during sedimentation in a low centrifugal field, or by measuring under the microscope the unloaded shortening of individual fibrils. The prevention of the mechanical change induced by adenosine triphosphate is generally assumed to be a phenomenon similar to relaxation.

In the present communication we wish to report that transphosphorylation is a very sensitive regulator of muscle 'relaxation' even in the Marsh experiment. This is noticeable particularly if the transphosphorylating enzyme systems are used in conjunction with such minimal amounts of microsomal component as would produce alone scarcely any effect. Figs. 1, 2 and 3 show this striking potentiation by the creatine phosphate and phosphoenolpyruvate kinase systems for inhibiting contraction. During centrifugation at about 1,000 r.p.m., at room temperature, the volume of the myofibrillar layer was measured with the aid of stroboscopic light. In Fig. 3 the results are presented in a somewhat more representative fashion. Fractional 'relaxation' (*Q*) is defined in terms of the volumes of myofibrillar layers as:

$$Q = \frac{V - V\ (\text{contracted})}{V\ (\text{standard}) - V\ (\text{contracted})} \times 100 \quad (1)$$

where V represents an experimental volume at time t, V (standard) is the volume at time t of myofibrils sedimenting without the addition of adenosine triphosphate, and V (contracted) stands for the final volume of the myofibrillar pellet sedimented in the presence of adenosine triphosphate. It will be seen that values of Q less than 100 indicate contraction, and it can also be noted that both the creatine phosphate and phosphoenolpyruvate kinase systems prevent changes in volume very effectively under the experimental conditions described. Though not shown on the graphs, we have further demonstrated that the extent to which contraction is inhibited appears to be, within certain limits, a function of the concentration of phosphagens and their enzymes. Actually, by selecting appropriate conditions, Q values considerably greater than 100 can be obtained, which signify a swelling of the myofibrils.

Since lowering of the myofibrillar adenosine triphosphatase activity appears to be a characteristic symptom of the relaxed state in muscle (see, for example, ref. 13), we have studied the rate of hydrolysis of adenosine triphosphate by the myofibrils in the presence of various combinations of the microsomal component and transphosphorylating enzyme systems. Fig. 4 illustrates the finding that adenosine triphosphatase, though not suppressed by the concentrations employed of microsomes or transphosphorylase and phosphagen alone, is effectively reduced if they are all present simultaneously.

The myofibrillar material used in these experiments was obtained by homogenizing minced rabbit muscle with 0·15 *M* potassium chloride (buffered to about *p*H 6·8 with potassium salts of veronal and acetate) for 3 min. in the cold in a 'Virtis' homogenizer at 30,000 r.p.m. After centrifuging at 3,000 r.p.m., the light upper fibrillar layer was separated from the cell debris and was resuspended repeatedly (6–10 times) with the aid of the homogenizer and washed in buffered potassium chloride. In many instances, the fibrils were stored at − 20° C. in 50 per cent (v/v) glycerol and washed again in potassium chloride several times before use to eliminate the glycerol.

The microsomal component was isolated from the muscle homogenate as the fraction sedimenting between 11,000 and 54,000*g*, after the elimination of the inactive sediment obtained during centrifugation at 11,000*g* for 30 min. The microsomal fraction was resuspended in potassium chloride with

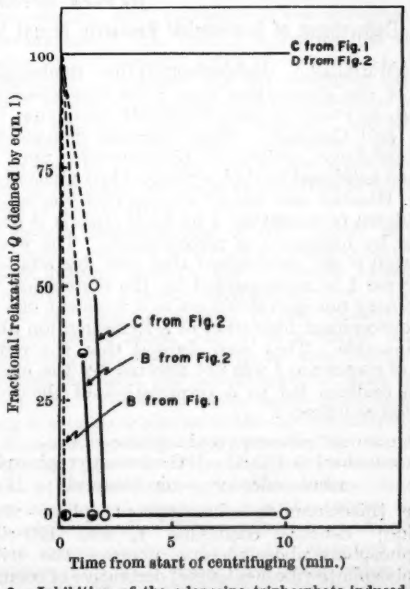

C from Fig. 1
D from Fig. 2
Fractional 'relaxation' Q (defined by eqn. 1)
C from Fig. 2
B from Fig. 2
B from Fig. 1
Time from start of centrifuging (min.)

Fig. 3. Inhibition of the adenosine triphosphate-induced contraction of myofibrils by the creatine phosphate and phosphoenolpyruvate kinase systems. For explanation see text

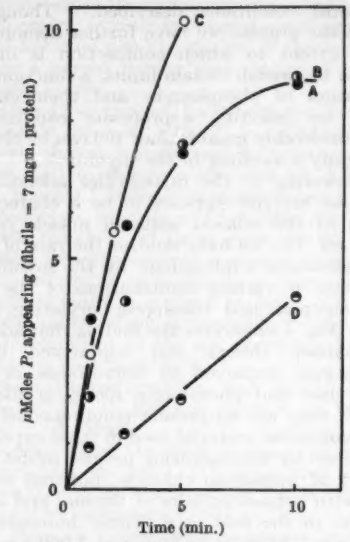


Fig. 4. Reduction of myofibrillar adenosine triphosphatase under the joint influence of microsomes and the phosphoenolpyruvate kinase system. Inorganic phosphate was measured by the method of Fiske and SubbaRow (ref. 20). Reaction mixture of 2·5 ml. at 25° C.: *p*H 6·5; $\mu \approx 0{\cdot}15$; 4 m*M* magnesium chloride; 4 m*M* adenosine triphosphate; about 7 protein-mgm. myofibrils. *A*, fibrils alone; *B*, fibrils + 1·19 protein-mgm. microsomes; *C*, fibrils + 0·35 protein-mgm. microsomes + 2·5 m*M* phosphoenolpyruvate + 150 μgm. phosphoenolpyruvate kinase; *D*, fibrils + 1·19 protein-mgm. microsomes + 2·5 m*M* phosphoenolpyruvate + 150 μgm. phosphoenolpyruvate kinase

the aid of a Potter hand-operated ground-glass homogenizer and centrifuged as before. This washing procedure was repeated several times.

In conclusion, the experiments presented here show that the interaction of the microsomal component of muscle with a transphosphorylating system is an essential feature of relaxation.

This work was aided by a grant from the Muscular Dystrophy Associations of America, Inc.

1 Szent-Györgyi, A., *Biol. Bull.*, **96**, 140 (1949).
2 Lorand, L., *Nature*, **172**, 1181 (1953). Goodall, M. C., and Szent-Györgyi, A. G., *Nature*, **172**, 84 (1953).
3 Moos, C., and Lorand, L., *Biochim. Biophys. Acta*, **24**, 461 (1957).
4 Lorand, L., Molnar, J., and Moos, C., *Biol. Bull.*, **113**, 323 (1957).
5 Lorand, L., Molnar, J., and Moos, C., Conf. Chemistry of Muscular Contraction, Igaku Shoin, Ltd., Tokyo, Japan, 85 (1957).
6 Kumagai, H., Ebashi, S., and Takeda, F., *Nature*, **176**, 166 (1955).
7 Ebashi, S., Conf. Chemistry of Muscular Contraction, Igaku Shoin, Ltd., Tokyo, Japan, 89 (1957).
8 Ebashi, S., *Arch. Biochem. Biophys.*, **76**, 410 (1958).
9 Weber, H. H., "Ann. Rev. Biochim.", **26**, 673 (1957).
10 Briggs, F. N., and Portzehl, H., *Biochim. Biophys. Acta*, **24**, 482 (1957).
11 Portzehl, H., *Biochim. Biophys. Acta*, **24**, 474 (1957).
12 Gergely, J., Briggs, F. N., and Kaldor, G., Fourth Internat. Congr. of Biochem. Abstracts of Communications, 83 (1958). Gergely, J., *Ann. N.Y. Acad. Sci.*, **72**, 546 (1959).
13 Bendall, J. R., *Nature*, **181**, 1188 (1958).
14 Marsh, B. B., *Biochim. Biophys. Acta*, **9**, 247 (1952).
15 Hirsch, A., and Cattaneo, C., *Physiol. Chem.*, **304**, 53 (1956).
16 Baer, E., in Ball, E., "Biochemical Preparations", **2**, 25 (J. Wiley and Sons, Inc., New York, 1952).
17 Bucher, T., and Pfleiderer, G., in "Methods in Enzymology", **1**, 435 (Academic Press, New York, 1955).
18 Ennor, A. H., and Stocken, L. A., *Biochem. J.*, **43**, 190 (1948).
19 Kuby, S. A., Noda, L., and Lardy, H. A., *J. Biol. Chem.*, **209**, 191 (1954).
20 Fiske, C. H., and SubbaRow, Y., *J. Biol. Chem.*, **66**, 375 (1925).

MECHANISM OF ACTION OF DIPHOSPHOPYRIDINE NUCLEOTIDASES

By Prof. SPYRIDON G. A. ALIVISATOS

Department of Biochemical Research, Mount Sinai Medical Research Foundation, Chicago Medical School

MAMMALIAN diphosphopyridine nucleotidases of the glycosidase type were first clearly described, as enzymes that inactivate coenzyme 1, by Mann and Quastel[1]. These workers demonstrated the inhibitory action of nicotinamide upon the reaction catalysed by diphosphopyridine nucleotidase. Later, Handler and Klein[2] showed that the enzymic breakdown of coenzyme 1 by tissue debris is accompanied by liberation of nicotinamide. The work of McIlwain *et al.*[3] established that this degradation of coenzyme 1 is accompanied by the conversion of a quaternary nitrogen structure to a trivalent one with the concomitant liberation of a hydrogen ion and of nicotinamide. They also showed that the reduced form of coenzyme 1 was not affected by the enzyme. These findings led to a formulation of the overall reaction as follows :

[nicotinamide$^+$·ribose-pyrophosphate-ribose-adenine] + HO·H → HO·ribose-pyrophosphate-ribose-adenine + nicotinamide + H$^+$ (1)

where [nicotinamide$^+$·ribose-pyrophosphate-ribose-adenine] denotes coenzyme 1, and HO·ribose-pyrophosphate-ribose-adenine denotes the (ribose-pyrophosphate-ribose-adenine) derivative of coenzyme 1 after its nicotinamide moiety has been replaced by a hydroxyl-group.

Later, Zatman *et al.*[4] found that mammalian diphosphopyridine nucleotidase also catalyses an exchange reaction between free nicotinamide and the nicotinamide moiety of coenzyme 1. An explanation of this phenomenon was offered by Zatman *et al.*[4] by postulating a two-step reaction :

[nicotinamide$^+$·ribose-pyrophosphate-ribose-adenine] + enzyme ⇌ [enzyme$^+$·ribose-pyrophosphate-ribose-adenine] + nicotinamide (2)

[enzyme$^+$·ribose-pyrophosphate-ribose-adenine) + HO·H → HO·ribose-pyrophosphate-ribose-adenine + enzyme + H$^+$ (3)

Alivisatos *et al.*[5] found a diphosphopyridine nucleotidase in rabbit erythrocytes and described its distribution in these cells. They[6] also studied some of the requirements for the attachment of the substrate (coenzyme 1) on the enzyme. Later, Alivisatos and Woolley[7] solubilized and purified the diphosphopyridine nucleotidase from beef spleen, and, following a hypothesis of Woolley[8] on the utilization of the free energy of hydrolysis of 'onium' linkages, Alivisatos and Woolley[9] found that 5-amino-4-carboxamido-imidazole reacted with coenzyme 1 to form a new dinucleotide. In contrast to exchanges (for example, as in reaction 2), this imidazolytic[10] breakdown of

coenzyme 1 was characterized by its irreversibility; the liberation of a hydrogen ion; and the presence of a trivalent nitrogen structure in the imidazole-ribose bond of the product[9]. The latter characteristic was most clearly demonstrated by the utilization of the new dinucleotide as a substrate in the enzymic formation of inosinic acid[10]. This demonstration established the identity of the imidazole-ribose bond in the dinucleotide with the imidazole-ribose bond present in the well-known precursor of purines, 5-amino-4-carboxamido-imidazole ribotide. Since the base-to-sugar configuration in coenzyme 1, in 5-amino-4-carboxamido-imidazole ribotide and in inosinic acid is the same (namely, β), the above-mentioned identity[10] implied a retention of configuration during the imidazolytic process and pointed towards a two-step reaction, each involving a Walden inversion[11]. The first step should be identical to the first step encountered in hydrolytic processes (reaction 2). This would also account for the inhibitory effect of nicotinamide upon the imidazolytic breakdown. The second step could be summarized in reaction (4), which should be taken as an alternative of reaction (3), if the imidazole is present in the medium:

[enzyme⁺·ribose-pyrophosphate-ribose-adenine] + imidazole → enzyme + imidazole·ribose-pyrophosphate-ribose-adenine + H⁺ (4)

where imidazole denotes the 5-amino-4-carboxamido-imidazole and other symbols have the same meaning as in reaction (1).

The finding[12] that another imidazole, namely, histamine, is even more capable of participating in reaction (4) focused new interest on the reactions catalysed by diphosphopyridine nucleotidase and called for a unified theory of action of this enzyme. (The "dinucleotide II" mentioned in a previous publication[12] proved later to be an artefact arising during the isolation procedures from "dinucleotide I". The imidazole-ribose bond of the latter has a trivalent nitrogen structure at *p*H 7·0, but it acquires a proton at lower *p*H values (*pK'* about 4·0).)

The above-mentioned observations, together with recent preliminary experimental evidence mentioned in a later part of the article, led to the formulation of the following hypothesis concerning the identity and the catalytic mechanism of the active group(s) of mammalian diphosphopyridine nucleotidases of the glycosidase type:

The two steps encountered in both the hydrolytic and imidazolytic processes (reactions (2), (3) and (4)) are virtually a succession of two nucleophilic attacks (each involving an inversion) upon carbon 1′ of the ribose moiety of coenzyme 1 close to nicotinamide. In the first (common) step the nucleophilic agent is a residue on the enzyme, while in the second step this part is played by water (hydrolysis) or an imidazole (imidazolysis).

While various groups of the diphosphopyridine nucleotidase may be important for its catalytic activity, our present evidence (see below) favours the participation of its histidyl-moieties. Of the possible forms of the amidine part of histidyl groups on the enzyme, the non-ionized form is expected to predominate over the *p*H range of activity of diphosphopyridine nucleotidases[6]. Certain contribution to the catalytic activity may possibly come from the anionic (deprotonated) form, especially at high *p*H values[13]. However, for clarity of discussion, only the non-ionized form is illustrated in Fig. 1 as participating in the catalysis. Thus, provided that the

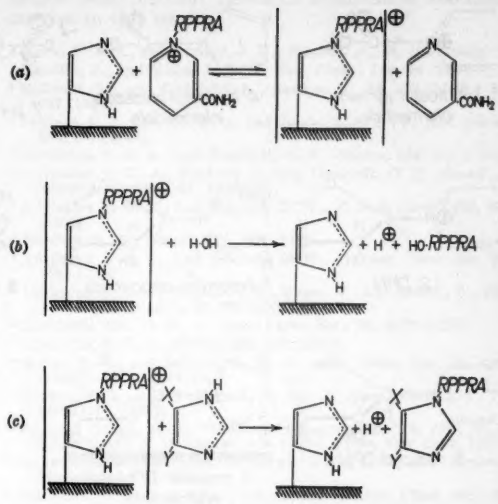


Fig. 1. Proposed scheme for the diphosphopyridine nucleotidase activities: *a*, exchange; *b*, hydrolysis; *c*, imidazolysis. The extreme (reactive) forms of histidyl groups of the free enzyme would be those of dipoles. *X* and *Y*, various radicals; RPPRA, ribose-pyrophosphate-ribose-adenine

proper requirements for attachment of the substrate (coenzyme 1) on the enzyme surface are fulfilled[6], nicotinamide may be displaced from coenzyme I by a histidyl group of the enzyme (Fig. 1, *a*). The structures in brackets in Fig. 1 correspond to the [enzyme⁺·ribose-pyrophosphate-ribose-adenine] intermediate encountered in reactions (2), (3) and (4).

In the absence of an excess of nicotinamide, other nucleophilic agents, for example, a hydroxyl or an imino-nitrogen of an imidazole, may react with the intermediate (Fig. 1, reactions *b* and *c*). The irreversibility of reactions *b* and *c* is due to the absence of an 'onium' structure in the products (HO·ribose-pyrophosphate-ribose-adenine and imidazole·ribose-pyrophosphate-ribose-adenine). An 'onium' structure like the one present in the oxidized form of coenzyme 1 (Fig. 1, reaction *a*) is necessary for the reversible exchange of the ·ribose-pyrophosphate-ribose-adenine radical with the enzyme. Thermodynamically, the necessity for a quaternary nitrogen structure reflects the higher free energy of hydrolysis of the nicotinamide-ribose bond, as compared with the free energy of hydrolysis of the imidazole-ribose bond[10].

A deeper understanding of the 'onium' structure requirement may be achieved by comparison with a simpler system, namely, the imidazole catalysis of hydrolysis of *p*-nitrophenyl-acetate[13,14]. In that case, a low electron density in the vicinity of the carbonyl-carbon atom (Fig. 2) is a major factor in determining the susceptibility of this carbon to a nucleophilic attack (for a discussion of other factors, see ref. 13). The nucleophilic agent is an imidazole, acting in a manner similar to the one postulated above for diphosphopyridine nucleotidase (Fig. 1). In the reaction catalysed by diphosphopyridine nucleotidase, on the other hand, the glycosidic carbon atom of a ribose moiety of coenzyme 1 is the target for the nucleophilic attack. This carbon is rendered to a certain extent electrophilic by the attached 'onium' structure (Fig. 2). While this polarization of the nicotinamide-ribose bond is apparently insufficient to make coenzyme 1 a suitable substrate for an

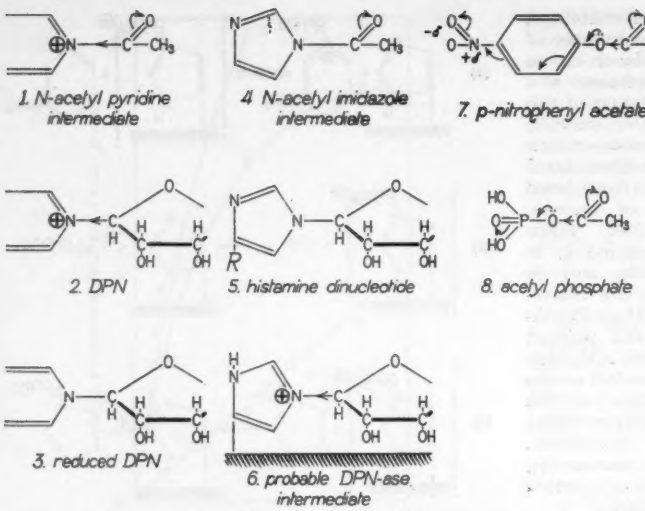


Fig. 2. Substrates, intermediates and end-products involved in reactions catalysed by pyridine, imidazole and diphosphopyridine nucleotidase

imidazole-catalysed hydrolysis[12], imidazolyl groups of the enzyme are probably capable of serving this purpose. A study of the influence of the various groups attached upon the carbon atom which serves as the electrophilic centre in substrates and intermediates (Fig. 2) involved in the hydrolytic processes catalysed by pyridine[11], imidazole[13,14] and the diphosphopyridine nucleotidase, will clarify this point better. Of the three substrates involved in these hydrolytic processes, namely, acetyl-phosphate, *p*-nitrophenyl-acetate and coenzyme I respectively, the latter is the most stable to spontaneous hydrolysis. This is due to the presence of a glycosidic structure, instead of a carbonyl, in the susceptible site of coenzyme I. On the other hand, all three intermediates, namely, N-acetyl-pyridinium, N-acetyl-imidazole and the [enzyme$^+$·ribose-pyrophosphate-ribose-adenine], hydrolyse spontaneously after their formation. However, N-acetyl-pyridinium[11] has a much shorter life than N-acetyl-imidazole[14] in water. This is due to the presence of an 'onium' structure in the former intermediate[14]. The importance of an 'onium' structure in promoting the ability of such a bond to hydrolysis is further demonstrated by the well-known higher stability of the reduced form of coenzyme I to alkaline hydrolysis, as compared to its oxidized form (Fig. 2). The lability of the N(ring)-acetyl-histamine intermediate[13], as compared to the high stability of the imidazole-ribose bond in the histamine dinucleotide (Fig. 2), illustrates the importance of a carbonyl structure or other means tending to intensify the electrophilic character of the carbon atom at the site of the nucleophilic attack.

By analogy with the above examples, the extreme lability of the [enzyme$^+$·ribose-pyrophosphate-ribose-adenine] intermediate is probably due to a concerted action of an 'onium' structure and of other groups on the enzyme surface tending to increase the electrophilic character of the glycosidic carbon atom. The problem of the nature of the latter groups is left, at present, open.

The availability of soluble purified diphosphopyridine nucleotidase[7] prompted a more direct experimental approach to the problem of the identity of the active centre(s) of the enzyme. Although our evidence is as yet of a preliminary nature, it strongly favours the hypotheses put forward here. In each of these experiments 16 units of the enzyme (0·4 mgm. of protein) were treated, in a 5 per cent sodium bicarbonate medium (*p*H 8·0, 3·0 ml. total vol.), with the various reagents indicated in Table 1. When 2,4 : dinitrofluorobenzene was used, the reaction was carried in a heterogeneous system with 0·25 ml. of the reagent added. In this instance, the watery phase of both the experimental system and its control contained, in addition to bicarbonate, 5 per cent methanol. All other reagents were soluble, in the concentrations employed, in the bicarbonate medium. Proper controls (no reagent added) accompanied all experiments. The mixtures were shaken at a slow rate (prevention of frothing) at 4° C. for periods indicated in Table 1. At the end of the incubation, the samples were transferred in casings and dialysed, in separate containers, against 200 ml. of 5 per cent sodium bicarbonate, at 4° C. The apparatus allowed a continuous mixing of the contents of the casings as well as of the outside liquid in the containers. The latter was changed 4 times in a period of 48 hr. Hydrolytic diphosphopyridine nucleotidase activity was tested as previously[7].

As shown in Table 1, 2,4 : dinitrofluorobenzene completely abolished the enzymic activity of the sample. Photo-oxidation with methylene blue had the same effect. If —SH groups can be excluded on other grounds (see below), this effect of methylene blue is regarded as rather specific for the histidyl residues of a protein[15]. Fluorescein *iso*thiocyanate reacts primarily with free aliphatic amino-groups (for example, lysine). However, we found that it also reacts with imidazoles (for example, acetyl histamine). In accordance, it had an effect on the diphosphopyridine nucleotidase activity (Table 1). Azo-enzyme, prepared by reaction with 3·75 μmoles of diazotized sulphanilic acid, was completely inactive. However, a ten times smaller amount of the reagent resulted in only partial inactivation. These results indicate reaction first with tyrosyl residues and, afterwards, with histidyl groups of the protein. However, anomalies in the interaction of diazo-

Table 1. Effect of various Reagents upon the Activity of Diphosphopyridine Nucleotidase

Reagent	Total amount of reagent (μmol.)	Duration of reaction (hr.)	Per cent loss of hydrolytic activity
1. 2,4 : Dinitrofluorobenzene	excess	2	100
2. Methylene blue (light)	1·400	6	100
3. Methylene blue (darkness)	1·400	6	9
4. Fluorescein *iso*thiocyanate	2·500	2	25
5. Fluorescein *iso*thiocyanate	2·500	16	56
6. Diazotized sulphanilic acid	0·375	16	45
7. Diazotized sulphanilic acid	3·750	16	100
8. Iodine (in 0·1 *M* potassium iodide)	0·500	6	14
9. Iodine (in 0·1 *M* potassium iodide)	10·000	6	100
10. Iodoacetate	5·000	16	0
11. *p*-Chloromercuribenzoate	0·320	6	0
12. *p*-Chloromercuribenzoate	6·500	6	0

reagents with proteins should be kept in mind[16]. The effect of iodine (Table 1) may be interpreted in a similar manner. Iodoacetate and *p*-chloromercuribenzoate did not have any appreciable effect on the activity of diphosphopyridine nucleotidase. The exclusion of —SH groups as possible participants in the enzymic activity was rather unexpected, in view of results by Stadtman *et al.*[17]. These workers discovered an enzyme that catalyses a reaction between acetyl-S-coenzyme *A* and imidazole to form the N-acetyl derivative.

Histidyl residues have been regarded as essential to the activity of several hydrolytic enzymes (see refs. in 13 and 14). According to our experimental findings, this seems to be also true for the diphosphopyridine nucleotidase. Considering the diverse catalytic capacities of this enzyme (namely, exchanges, hydrolytic and imidazolytic processes), that essentially parallel the various model systems developed for the study of the mechanism of enzymic hydrolysis, diphosphopyridine nucleotidase may prove very useful for such studies.

This project was partially supported by a U.S. National Institutes of Health grant No. *E*-2436. I wish to thank Drs. I. Davidsohn and K. Stern of the Mount Sinai Medical Research Foundation for their interest in this work.

[1] Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, **35**, 502 (1941).
[2] Handler, P., and Klein, J. R., *J. Biol. Chem.*, **143**, 49 (1942).
[3] McIlwain, H., and Rodnight, R., *Biochem. J.*, **44**, 470 (1949); **45**, 337 (1949).
[4] Zatman, L. J., Kaplan, N. O., and Colowick, S. P., *J. Biol. Chem.*, **200**, 197 (1953).
[5] Alivisatos, S. G. A., and Denstedt, O. F., *Science*, **114**, 281 (1951).
[6] Alivisatos, S. G. A., Kashket, S., and Denstedt, O. F., *Canad. J. Biochem. Physiol.*, **34**, 46 (1956).
[7] Alivisatos, S. G. A., and Woolley, D. W., *J. Biol. Chem.*, **219**, 823 (1956).
[8] Woolley, D. W., *Nature*, **171**, 323 (1953).
[9] Alivisatos, S. G. A., and Woolley, D. W., *J. Amer. Chem. Soc.*, **77**, 1065 (1955).
[10] Alivisatos, S. G. A., LaMantia, L., Ungar, F., and Savich, B., *Biochim. Biophys. Acta*, **30**, 660 (1958).
[11] Koshland, jun., D. E., *J. Amer. Chem. Soc.*, **74**, 2286 (1952).
[12] Alivisatos, S. G. A., *Nature*, **181**, 271 (1958).
[13] Bruice, T. C., and Schmir, G. L., *J. Amer. Chem. Soc.*, **79**, 1663 (1957); **80**, 148 (1958).
[14] Bender, M. L., and Turnquest, B. W., *J. Amer. Chem. Soc.*, **79**, 1652, 1656 (1957).
[15] Fraenkel-Conrat, H., in "Methods in Enzymology", **4**, 247; S. P. Colowick and N. O. Kaplan, eds. (Acad. Press, New York, 1957).
[16] Gelewitz, E. W., Riedeman, W. L., and Klotz, I. M., *Arch. Biochim. Biophys.*, **53**, 411 (1954).
[17] Stadtman, E. R., and White, jun., F. H., *J. Amer. Chem. Soc.*, **75**, 2022 (1953).

MOLECULAR ADAPTATION IN HÆMOGLOBINS: NATURE OF THE BOHR EFFECT

By AUSTEN RIGGS
Department of Zoology, University of Texas, Austin

SCHMIDT-NIELSEN[1] has shown that among terrestrial mammals the smaller the animal the higher is the oxygen pressure (P_{50}) at which the whole blood is half-saturated with oxygen. Thus the blood is adapted to deliver oxygen to the tissues at higher pressures in the smaller animals the specific rates of oxygen consumption of which are highest.

Recently, I have found[2] that the primary difference between these bloods lies in the sensitivity of their hæmoglobins to changes in *p*H. As the 'Bohr effect', it is well known that high carbon dioxide pressure or decreased *p*H tends to dissociate oxyhæmoglobin, and so promotes gas exchange in the tissues. In a series of terrestrial mammals, ranging in size from elephant to mouse, the smaller the animal the greater is the sensitivity of the oxygen equilibrium of its hæmoglobin to *p*H. This *p*H sensitivity (Bohr effect) reflects the fact that certain acid groups of the hæmoglobin are functionally linked to the oxygenation process in such a way that they become more acidic when oxygen is bound[3,4]. Therefore, the results indicate that the smaller the mammal the greater is the number of protons discharged from its hæmoglobin when the molecule is oxygenated. This effect not only promotes gas exchange but also is a homœostatic buffering mechanism.

I have now determined the number of mercuric ions which can be immediately bound to these hæmoglobins, using the amperometric procedure of Kolthoff, Stricks and Morren[5]. The hæmoglobin solutions were prepared as previously described[6]. The titrations were performed at 25° C. in an atmosphere of helium; thus reduced hæmoglobin was titrated. These titrations indicate a linear relation between the magnitude of the Bohr effect, defined as $\Delta \log P_{50}/\Delta p\mathrm{H}$, and the number of mercuric ions bound per molecule of hæmoglobin. This is shown in Fig. 1. Only the rabbit appears really exceptional.

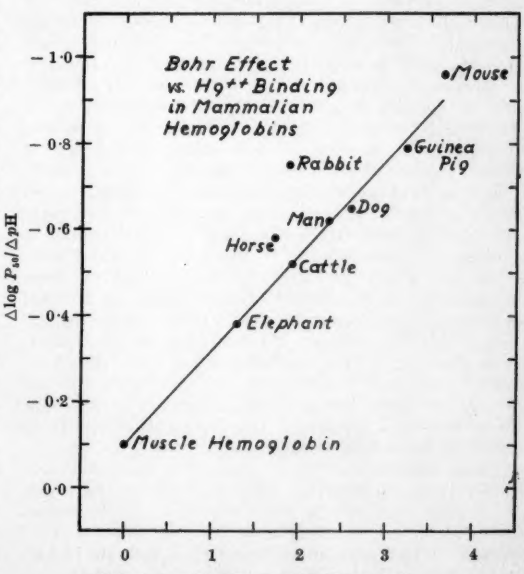


Fig. 1. The relation between the magnitude of the Bohr effect and the number of mercuric ions which can be bound per hæmoglobin molecule. The data for muscle hæmoglobin are from Theorell (ref. 8)

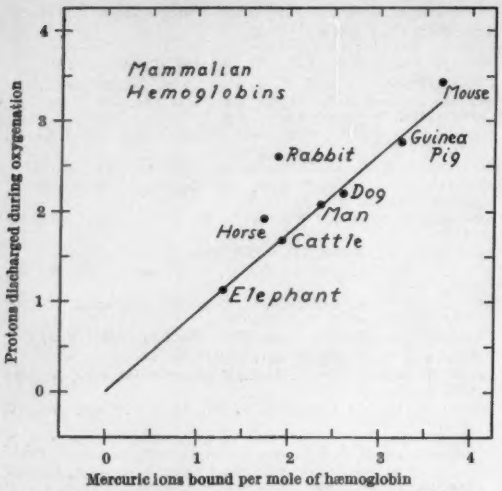


Fig. 2. The relation between the number of protons discharged by hæmoglobin during its oxygenation as calculated in the text, and the number of mercuric ions which may be bound per molecule

Thus the magnitude of the Bohr effect appears to be directly proportional to the number of sulphydryl groups.

The conclusion that the oxygen-linked acid groups are sulphydryl groups is strengthened by the following considerations. Horse-muscle hæmoglobin has no sulphydryl groups[7]; the value of its Bohr effect[8] is approximately 0·1. Extrapolation of the line in Fig. 1 to the point at which zero mercuric ions are bound corresponds to just this value. If we assume that the small non-sulphydryl Bohr effect exhibited by muscle hæmoglobin is also present in all the blood hæmoglobins, then we can subtract the Bohr effect value in muscle hæmoglobin from all the other values to obtain the Bohr effect due solely to sulphydryl groups. Wyman has shown that $\Delta \log P_{50}/\Delta p\mathrm{H}$ is directly proportional to the number of protons discharged during oxygenation[4]. Therefore we should be able to calculate the number of sulphydryl protons dissociated during oxygenation. In Fig. 2 the number of protons so calculated is plotted against the number of mercuric ions bound. It is clear that a rather good one-to-one quantitative correspondence exists. The number of mercuric ions bound is only 10 per cent higher than the number of protons dissociated. If the oxygen-linked acid groups are sulphydryl groups, the binding of these groups with an irreversible reagent such as N-ethyl maleimide should result in greatly reducing the magnitude of the Bohr effect. We find that low concentrations (5 moles N-ethyl maleimide per mole of hæmoglobin) have just this effect: not only is the oxygen affinity increased but also the magnitude of the Bohr effect decreases greatly: $\Delta \log P_{50}/\Delta p\mathrm{H}$ drops from 0·65 to 0·28 in human hæmoglobin at 35° C.

These observations led us to predict that whenever we find hæmoglobins the oxygen equilibria of which are insensitive to pH (no Bohr effect), we might expect to find either no, or a very small number of, sulphydryl groups. I had previously found that tadpole hæmoglobin has no Bohr effect whereas that of the adult bullfrog has a large Bohr effect[5]. I have re-examined tadpole hæmoglobin, and find that it binds the smallest number of mercuric ions of any blood hæmoglobin so far examined: only 0·8 mercuric ion per mole of hæmoglobin. In contrast, the adult bullfrog hæmoglobin molecule binds about 4·2 mercuric ions.

It is well known that the four hæm groups present in each hæmoglobin molecule interact with one another. That is, oxygenation of certain of these hæms greatly enhances the affinity of other hæms for oxygen. I have shown previously that sulphydryl reagents can abolish this interaction[6–10]. It now appears that the interaction is destroyed when mercurials are bound to the oxygen-linked acid groups which are now identified as sulphydryl groups. Thus two properties of hæmoglobin of great physiological importance, the S-shape of the oxygen equilibrium curve and the dependence of the equilibrium on pH, are both mediated by sulphydryl groups.

The suggestion that the oxygen-linked acid groups are imidazole residues was based on the correspondence between the observed pK values and heats of dissociation and those of imidazole[4], and on the changes anticipated in the electronic structure of imidazole resulting from covalent linkage with the hæm iron[11]. Unfortunately, both the pK values and the heats of dissociation are often very similar in both —SH groups and imidazole[12,13]. Although mercurials do have affinity for imidazole groups, the affinity for —SH groups is generally much higher. Furthermore, in human hæmoglobin at least, a one-to-one correspondence exists between the number of N-ethyl maleimide molecules bound, and the number of cysteine residues found analytically[14]. In our experiments the number of mercuric ions that can be immediately bound by human hæmoglobin drops by two-thirds within 5 min. after the addition of five moles of N-ethyl-maleimide per mole of hæmoglobin; the mercury titration is zero after 15 min. The effect of N-ethyl-maleimide on the oxygen equilibrium is very similar to that of the mercurial, mersalyl.

These observations suggest that one of the primary physiological adaptations of hæmoglobins is brought about by varying the number of cysteine residues per molecule. If it is physiologically advantageous for an animal to possess a hæmoglobin with a large Bohr effect, mutants with hæmoglobins possessing a larger number of appropriately placed cysteine residues would be selected. This conclusion is supported by the amino-acid analyses of Birkofer and Taurins[15], who examined the hæmoglobins of 7 species of mammal. Their results clearly indicate an inverse relation between body-weight and number of cysteine residues in each hæmoglobin.

A full report of these experiments will be published elsewhere. I wish to acknowledge the assistance of Messrs. Patrick Barlow and Allen Tyler. The research was supported in part by a grant from the National Science Foundation.

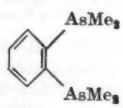
1 Schmidt-Nielsen, K., and Gjönnes, B., *Fed. Proc.*, **11**, 140 (1952).
2 Riggs, A., and Tyler, A., *Fed. Proc.*, **17**, 297 (1958).
3 Henderson, L. J., *J. Biol. Chem.*, **41**, 401 (1920).
4 Wyman, J., "Adv. in Protein Chem.", **4**, 407 (1948).
5 Kolthoff, I. M., Stricks, W., and Morren, L., *Anal. Chem.*, **26**, 366 (1954).
6 Riggs, A., and Wolbach, R. A., *J. Gen. Physiol.*, **39**, 585 (1956).
7 Tristram, G. R., in "Hæmoglobin", ed. by F. J. W. Roughton and J. C. Kendrew, 109 (Interscience Publishers, New York, 1949).
8 Theorell, H., *Biochem. Z.*, **268**, 73 (1934).
9 Riggs, A., *J. Gen. Physiol.*, **35**, 23 (1951).
10 Riggs, A., *J. Gen. Physiol.*, **36**, 1 (1952).
11 Coryell, C. D., and Pauling, L., *J. Biol. Chem.*, **132**, 769 (1940).
12 Benesch, R. E., and Benesch, R., *J. Amer. Chem. Soc.*, **77**, 5877 (1955).
13 Barnard, E. A., and Stein, W. D., "Adv. in Enzymol.", **20**, 51 (1958).
14 Cole, R. D., Stein, W. D., and Moore, S., *J. Biol. Chem.* (in the press).
15 Birkofer, L., and Taurins, A., *Z. physiol. Chem.*, **265**, 94 (1940).

LETTERS TO THE EDITORS

CHEMISTRY

New Oxidation States of Technetium

THE chemistry of technetium, the middle member of the Group VII triad with manganese and rhenium as its congeners, has been little investigated until recently owing to its rarity. Indeed, since the isolation of the isotope technetium-99 in gram quantities from the products of uranium fission[1] the oxidation states +7 and +4 only have been definitely established for this element, at least in solid compounds. These are exemplified by $KTcO_4$ and Tc_2S_7, in the first case and TcO_2, TcS_2, $TcCl_4$ and K_2TcHal_6 (Hal = Cl, Br and I) in the second[1]. Evidence[2] for the existence of green tervalent technetium in solution has been adduced from the reducing power of an electrolytically produced aqueous solution, but no compound of technetium III was isolated. Similarly, technetium V probably exists in solution as the complex thiocyanate, but no solid compound has been isolated[3]. The close similarity of rhenium and technetium suggested that for the preparation of lower oxidation states of technetium a study of the corresponding rhenium chemistry would be very helpful.



Ditertiary arsine (*D*)

Recent work[4] has shown that very stable bivalent and tervalent rhenium compounds may be prepared by using the ditertiary arsine (*D*) as a chelating ligand. These have the general formula $[Re^{II}D_2X_2]^0$ and $[Re^{III}D_2X_2]ClO_4$, where X = Cl, Br or I. The bivalent complexes are non-electrolytes, paramagnetic, ($\mu \simeq 1{\cdot}8$–$1{\cdot}9$ Bohr magnetons) and are clearly octahedral, spin-paired d_ε^6 compounds. The tervalent derivatives are uni-univalent electrolytes in nitromethane and clearly involve octahedrally co-ordinated rhenium III with a d_ε^4 configuration. The magnetic moments ($\simeq 1{\cdot}9$ Bohr magnetons) are less than the spin-only value for two unpaired electrons (2·83 Bohr magnetons), but these are readily understood in terms of the Kotani[5] theory for octahedral strong-field complexes of elements with a large spin-orbit coupling constant.

Following the procedure used to obtain the above rhenium compounds, we have now been able to prepare the corresponding technetium II and technetium III derivatives. Starting from potassium hexachlorotechnetate[6], the sequence of reactions employed is as follows:

Having only 15 mgm. of technetium, the usual analytical procedures are not feasible, but reactions 2 and 3 have been confirmed quantitatively by measuring both the amount of the less electronegative halide entering the complex and the amount of more electronegative halide which is displaced. Reaction 5 has been shown to proceed with the uptake of exactly one equivalent of iodine. Conductivity, molecular weight and magnetic data have been obtained on these compounds and, taken in conjunction with the known properties of the corresponding Re II and III derivatives, provide further confirmation of the composition and structure of the new complexes. Finally, it has been shown by X-ray powder photographs that the rhenium II and technetium II compounds are isomorphous. The properties of these compounds are summarized in Table 1.

Table 1. COMPOUNDS OF TECHNETIUM III and II

Oxidation state	Compound	Colour	$\mu_{eff.}$ (20°) B.M.	Spectra λ mμ	Spectra $\varepsilon \times 10^{-3}$	Molecular conductivity in nitromethane 25° C. $C = 1 \times 10^{-3} M$*
Tc^{III} (d^4)	$[TcD_2Cl_2]Cl$	Orange	2·7	441	3·92	75 ohm^{-1}
	$[TcD_2Br_2]Br$†	Red	3·2	472 302	5·56 3·08	77 ohm^{-1}
	$[TcD_2I_2]I_2$	Deep red to black	3·4	574 359 294	4·00 6·69 11·23	86 ohm^{-1}
Tc^{II} (d^5)	$[TcD_2I_2]^0$‡	Brown	~3·0	514 474	2·79 1·00	Slightly soluble but a non-electrolyte in Ph NO_2 and nitromethane

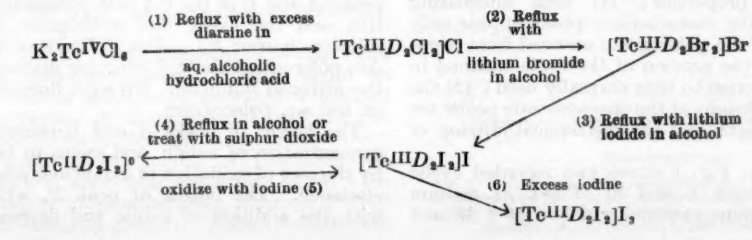
* Λ_M for a uni-univalent electrolyte in nitromethane at 10^{-3} *M* is 70–90 ohm^{-1}.

† Molecular weight. Found 435, isopiestic in acetone. Calculated for 1 – 1 electrolyte 455.

‡ From X-ray powder photographs the $[TcD_2I_2]^0$ is isomorphous with $[ReD_2I_2]^0$, and $[TcD_2I_2]I_2$ with $[ReD_2I_2]I_2$.

The tervalent technetium compounds are similar to those of tervalent rhenium, except that the magnetic moments are higher, as expected. They differ very much from the known[7] diarsine complexes of manganese III, which are spin-free and have the general formula $[MnDX_2.H_2O]ClO_4$, where X = Cl or Br.

Bivalent technetium resembles both manganese II and rhenium II in forming compounds of the type $[Tc^{II}D_2I_2]^0$, but the magnetic moment of this compound is unusual, agreeing neither with the spin-free value for five unpaired electrons as in $[MnD_2I_2]^0$, nor with the spin-paired value for one unpaired electron as in $[ReD_2I_2]^0$. The position of technetium in the periodic table provides a clue to this behaviour which



will be discussed in a more detailed report on these compounds to be published shortly.

We are grateful to the United Kingdom Atomic Energy Authority for the loan of the technetium used in this work. One of us (J. E. F.) also thanks the University of New Zealand for a postgraduate scholarship.

J. E. Fergusson
R. S. Nyholm

William Ramsay and
Ralph Forster Laboratories,
University College,
London.
March 20.

[1] Boyd, *J. Chem. Educ.*, **3**, 36 (1959). This paper gives full references to the chemistry of technetium and its compounds.
[2] Thomason, Oak Ridge Report ORNL 2453, 7 (1958). See also ref. 1.
[3] Crouthamel, *Anal. Chem.*, **29**, 1756 (1957).
[4] Curtis, Fergusson and Nyholm, *Chem. and Indust.*, 625 (1958). Also Fergusson and Nyholm, *ibid.*, 1555 (1958).
[5] Kotani, *J. Phys. Soc. Japan*, **4**, 293 (1949).
[6] Nelson, Boyd and Smith, *J. Amer. Chem. Soc.*, **76**, 348 (1954); **74**, 556 (1952). Dalziel, Gill, Nyholm and Peacock, *J. Chem. Soc.*, 4012 (1958).
[7] Nyholm and Sutton, *J. Chem. Soc.*, 564 (1958).

Cyclic Alternating-Current Voltammetry with a Platinum Electrode

The a.c. polarographic method of Brewer and Gutmann[1], in which a.c. instead of d.c. current is measured against polarization potential, has hitherto not been extended to the determination of compounds reducible or oxidizable at potentials more positive than the potential of dissolution of mercury. This limitation is due to the difficulty of reproducing a.c. current-voltage curves obtained with solid electrodes. Changes in the nature of the surface of the electrode are responsible for poor reproducibility in conventional voltammetry[2]; even worse results can be expected in a.c. voltammetry because changes in the nature of the electrode modify the impedance much more than the resistance of the double layer during the electrolytic process.

One can expect an electrode to acquire different reproducible states of accommodation during the electrolytic processes when submitted to a series of cyclic polarizations in a solution to be investigated. Kemula and Kublick[3] (and Kemula, K., personal communication) have already shown that perfectly repeatable d.c. polarograms are obtained when a hanging-drop mercury electrode is polarized cyclicly at a moderate rate between 0·2 and − 2·0 V. versus a standard calomel electrode. In fact, reproducible a.c. polarograms are also recorded with a platinum electrode when the electrode is polarized cyclicly at approximately 2·5 V./min. between − 1·0 and 1·5 V. versus a standard calomel electrode in a solution through which nitrogen is bubbling.

Besides their perfect repeatability, cyclic a.c. polarograms obtained with a platinum electrode have two important properties: (1) with alternating current most of the characteristic peaks appear only on the portion of the polarogram scanned from negative to positive, the portion of the cycle scanned in the opposite direction to that normally used; (2) the position and the height of the characteristic peaks are practically unaffected by the mechanical stirring of the solution.

As an example, Fig. 1 shows two recorded cyclic polarograms (curves 1 and 2) of 0·1 *M* sodium perchlorate solutions containing $2{\cdot}5 \times 10^{-4}$ *M* and 5×10^{-4} *M*, respectively, of potassium iodide, together with their d.c. polarograms (curves 1′ and 2′). These polarograms were obtained with a 3 mm.² platinum/10 per cent rhodium wire working electrode and a 25 cm.² saturated calomel auxiliary electrode. The a.c. polarograms were obtained by feeding the cell with a 60 c./s., 0·03 root mean square sinusoidal voltage, in parallel with the linearly scanned d.c. voltage. The a.c. and d.c. currents were restricted from other circuits by a low-resistance 10-henry inductor and by a capacitor of a few microfarads placed in the d.c. and a.c. circuits, respectively. The relative admittance of the cell is determined by the voltage drop across a resistor located in the a.c. circuit. This voltage was measured in these experiments with an electronic a.c. millivoltmeter, and was recorded through a self-balancing millivolt potentiometer.

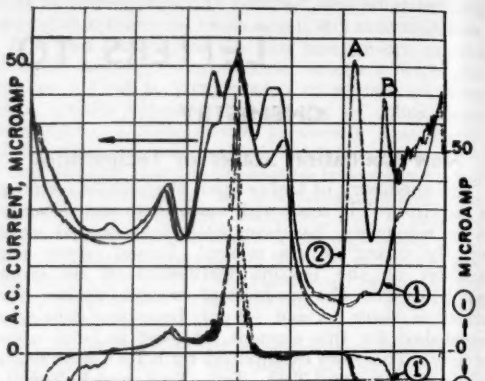


Fig. 1. Cyclic a.c. (curves 1 and 2) and d.c. (curves 1′ and 2′) polarograms of 0·1 *M* sodium perchlorate solution containing potassium iodide: (*a*) $2{\cdot}5 \times 10^{-4}$ *M* (curves 1 and 1′); (*b*) $5{\cdot}0 \times 10^{-4}$ *M* (curves 2 and 2′)

The addition of 0·5 m*M* potassium iodide to the 0·1 *M* sodium perchlorate solution (curves 2 and 2′) markedly affected the shape of the second half-cycle of the a.c. polarogram, while the shape of the part scanned from positive to negative of the curve remained practically unchanged. The repeatability of the curve is so good that there is scarcely any difference between the duplicated curves recorded in Fig. 1 despite the complicated pattern of the curve. No clear relation appeared between the admittance peaks *A* and *B* of the 0·5 m*M* potassium iodide solution and the cyclic d.c. polarogram of the same solution (curve 2′). The diffusion current of the d.c. polarogram showed irregular fluctuations due to the nitrogen bubbling. No such fluctuations appear on the a.c. polarogram.

The height of peaks *A* and *B* increases with the concentration of iodide, and seems to be determined by the rate of oxidation of iodide ions adsorbed on the electrode. The height of peak *B*, which increases with the addition of iodine and decreases with the

addition of starch or thiosulphate, seems to be related to the rate of oxidation of adsorbed iodine, probably to I^+. The amount of iodide and iodine contained in the solution can be determined by the height of the peaks *A* and *B* after establishment of a calibration curve.

Perfectly repeatable cyclic a.c. polarograms are also obtained when compounds like ferricyanide or hydroquinone, which are reversibly or semi-reversibly reducible or oxidizable between the potential of evolution of hydrogen and oxygen, are added to a sodium perchlorate solution. Repeatable polarograms are obtained even with species like Ag^+ and Pb^{2+}, which form a deposit on the surface of the electrode, when the solution is made acid with perchloric acid. In this case the results are repeatable so long as the amount of product reduced during the first half-cycle is still completely re-oxidized during the next one. The peak maximum obtained with deposited metal coincides quite well with the moment at which the last trace of the reduced product disappears at the surface of the electrode.

Cyclic a.c. voltammetry with a platinum electrode can therefore be used as a convenient method for the analysis of compounds reversibly or semi-reversibly reducible or oxidizable at potentials between $-1{\cdot}0$ and $+1{\cdot}5$ V. versus a standard calomel electrode. It also appears to be an excellent method for studying the mechanism of electrolytic processes at a solid electrode.

André L. Juliard

Analytical Laboratory,
Houdry Process Corporation,
Linwood, Pennsylvania.
Nov. 20.

[1] Brewer, B., and Gutman, F., *Austral. J. Sci.*, **8**, 21 (1945). For a general survey, Brewer, B., Gutman, F., and Baur, H. H., *Oesterreich. Chem. Ztg.*, **57**, 67 (1956).
[2] Kolthoff, I. M., and Tanaka, N., *Anal. Chem.*, **26**, 632 (1954).
[3] Kemula, W., and Kublick, Z., *Nature*, **182**, 793 (1958).

Polymorphic Conversion of Mercuric Iodide on Alumina

It is well known that mercuric iodide exists in two crystalline forms, the stable red form being converted to the metastable yellow at 127° C.[1]. Recently, Sieskind[2] has reported an orange modification which was isolated from both ethyl acetate and acetone.

The information available concerning the spectral characteristics of mercuric iodide in solution is apparently limited to a paper by Gewenlock and Trotman[3], who have reported that the transmission spectrum of red mercuric iodide in ethanol has two broad absorption bands in the ultra-violet, with maxima at approximately 215 and 273 mμ. No mention is made of any characterizing absorption in the visible region. There are no comparable results regarding the metastable yellow form which quickly reverts to the red form upon standing or treatment with solvents.

Work by Zeitlin and Niimoto[4] showing that characteristic reflexion spectra of solids could be obtained on active adsorbents provided a method for the measurement of the spectra of both the red and the yellow forms in the solid state.

A small quantity (50 mgm.) of powdered red mercuric iodide of analytical grade was mixed with 2·5 gm. of active chromatographic alumina (> 140 mesh) Woelm—activity grade 1. Within a short time it became evident that a conversion from the red to the yellow form was occurring. The change from a heterogeneously dispersed mixture of red mercuric iodide and alumina to one which was decidedly yellow and homogeneous in appearance was striking and practically complete in a few days at room temperature. The transition proceeded at 0° C. but at a much slower rate. It was substantially accelerated by mild heating. Although the yellow form is unstable when prepared in the usual manner, it apparently can be stabilized and maintained indefinitely on alumina in a moisture-free atmosphere. Exposure to the atmosphere or addition of water to the yellow form on alumina resulted in reversion to the red form. When the yellow form on alumina was extracted with ethanol or ether, the red modification was obtained.

The reflexion spectra of the solid mixtures were obtained with a Beckman *DU* spectrophotometer equipped with a standard reflectance attachment. Fig. 1, curves *a*, *b*, *c*, gives the spectra on alumina of the red, mixture of the red-yellow, and the yellow forms. The spectra, which are reproducible, differ in several details from the transmission spectra in ethanol. In reflectance, the first broad band is resolved into two smaller ones while the second maximum is retained. There is a definite bathochromic displacement of the optical peaks in going from transmittance to reflectance. In addition, there is a shoulder in the reflexion spectrum of the red form in the visible region which has not been reported in the transmission spectrum. In order to detect the spectral differences between the two forms, the spectra of the mixtures of mercuric iodide and alumina were taken immediately after mixing, after a few hours to permit partial conversion to the yellow modification, and after several days when the conversion to yellow mercuric iodide was complete. This enabled direct comparison of the spectra of the mixtures with identical quantities of mercuric iodide. The results show (Fig. 1, curves *a*, *b*, *c*), with increasing concentration of the yellow relative to the red form, a corresponding decrease in the absorbance in the shoulder region (about 560 mμ). The red form possesses in reflectance a shoulder in the visible that is absent in the yellow modification. It has been impossible to obtain the spectrum of the pure red form on alumina due to the change that takes place immediately after mixing. Consequently, red mercuric

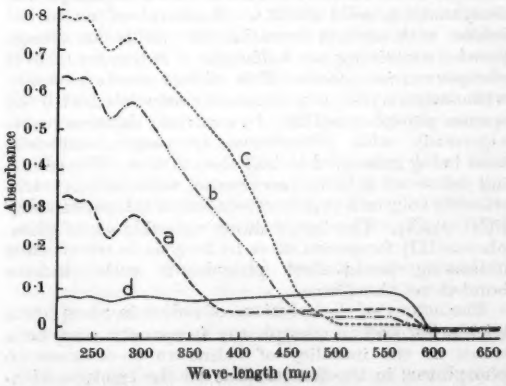


Fig. 1. Comparison of the reflexion spectra of polymorphic forms of mercuric iodide: *a*, red mercuric iodide on alumina; *b*, red-yellow mercuric iodide on alumina; *c*, yellow mercuric iodide on alumina; *d*, red mercuric iodide on sodium fluoride

iodide was mixed with powdered inactive sodium fluoride and the reflexion spectrum measured. The spectrum of pure red mercuric iodide (Fig. 1, curve *d*) so obtained is similar to the spectrum of the red form on alumina in all important details. The reflexion spectra of mercuric iodide on alumina are therefore not modified significantly by the active adsorbent.

Energy required for the conversion of the stable to the metastable form is apparently derived from the heat liberated by the adsorption of mercuric iodide on alumina. Our experimental findings are in agreement with the theory advanced by Tovbin and Baram[5] concerning the catalytic conversion of a stable to a less-stable form through heat of adsorption.

We gratefully acknowledge the financial support of the Research Corporation of New York provided by a Frederick Gardner Cottrell grant.

Harry Zeitlin
Harry Goya

Chemistry Department,
University of Hawaii,
Honolulu, Hawaii.
March 6.

[1] Kohlschutter, H. W., *Kolloidchem. Beihefte*, **24**, 319 (1927).
[2] Sieskind, M., *J. Chim. Phys.*, **55**, 661 (1958).
[3] Gewenlock, B., and Trotman, J., *J. Chem. Soc.*, 1454 (1955).
[4] Zeitlin, H., and Niimoto, A. (unpublished results).
[5] Tovbin, M., and Baram, O., *J. Ukrain. Khim. Zhur.*, **23**, 567 (1957).

Solution of Iodine in Phosphorus(III) *Isocyanate*

During an investigation of the solvent properties of phosphorus(III) *iso*cyanate[1] it was observed that iodine dissolved to give a reddish-brown solution. It is well known that such coloured solutions of iodine in 'active' solvents indicate some sort of solvent-solute interaction[2].

Previously[3], it had been noted that iodine dissolved in phosphorus trichloride to give a violet solution with an absorption maximum at 5000–5200 A. Since —NCO can be looked upon as a pseudo-halide, it might be expected that phosphorus *iso*cyanate and phosphorus trichloride would behave similarly in so far as reaction with iodine is concerned.

Iodine dissolves slowly in phosphorus *iso*cyanate with little heat of solution. The resulting solution is red-brown and shows an absorption maximum at 3300 A. If an excess of iodine is added to phosphorus *iso*cyanate a solid results. Removal of unreacted iodine with carbon tetrachloride yields an orange powder containing one half mole of iodine per mole of phosphorus *iso*cyanate. This adduct reacts violently with water, a property commonly associated with the species phosphorus(III). In contrast, chlorine reacts vigorously with phosphorus *iso*cyanate, sufficient heat being generated to boil the solution. The resulting yellow oil solidifies on contact with carbon tetrachloride to give a yellow crystalline solid, presumably $P(NCO)_2Cl_3$. The latter shows no evidence of phosphorus(III) formation after hydrolysis in water, thus indicating pentavalent phosphorus with chlorine bonded to phosphorus.

The colour of the solutions of iodine in phosphorus trichloride and in phosphorus *iso*cyanate may be a result of the inability of iodine to co-ordinate to phosphorus in the former (due to the electron-withdrawing power of chlorine) and the enhanced co-ordination in the latter (due to the electron donation of *iso*cyanate). Steric effects may also be involved in the case of iodine and phosphorus trichloride since no PCl_3I_2 has been confirmed, although such a compound has been reported[4].

The adducts $P(NCO)_3I$, as observed in this work, and PCl_3I [4] are similar in stoichiometry and would seem worthy of further investigation.

We wish to thank the Research Corporation of New York for financial support of part of this work.

Ervin Colton*
Lewis St. Cyr

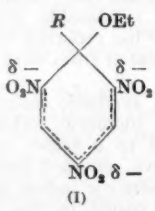
School of Chemistry,
Georgia Institute of Technology,
Atlanta, Georgia. Feb. 26.

* Present address: Research Division, Allis-Chalmers Mfg. Co., Milwaukee 1, Wis.

[1] Colton, E., and St. Cyr, L., *J. Inorg. Nucl. Chem.*, **7**, 424 (1958).
[2] Kleinberg, J., and Davidson, A. W., *Chem. Revs.*, **42**, 601 (1948).
[3] Colton, E., *J. Amer. Chem. Soc.*, **77**, 3211 (1955).
[4] Mellor, J. W., "A Comprehensive Treatise on Inorganic and Theoretical Chemistry", **8**, 1045 (Longmans, Green and Co., London, 1947).

Interaction of 1,3,5-Trinitrobenzene with Ethoxide Ion

When a dilute solution of 1,3,5-trinitrobenzene in ethanol is treated with sodium ethoxide, a yellow solution is formed. A similar colour is produced when sodium ethoxide is added to 2,4,6-trinitroanisole. The rates of development of the colours have been studied by Ainscough and Caldin[1], who found that there are two colour-producing reactions: (i) a 'fast' reaction the product of which they suggest is a charge-transfer complex, and (ii) a 'slow' reaction, which probably produces an addition compound where OEt is covalently bonded to the nucleus, as was suggested by Meisenheimer[2] for the solid obtained in this reaction (I, $R = OCH_3$). With 1,3,5,-trinitrobenzene and sodium ethoxide only one reaction was observed, and on the basis of similar rates of reaction and similar effects of acid on the two systems, Ainscough and Caldin suggested that the product is a charge-transfer complex analogous to the 'fast' reaction with 2,4,6-trinitroanisole. The absorption spectra of the trinitrobenzene + sodium ethoxide obtained by them did not resemble that of trinitroanisole + sodium ethoxide.



(I)

The absorption spectrum of 2,4,6-trinitroanisole in ethanol in the visible region in the presence of an excess of potassium ethoxide has been re-measured and compared with that of 1,3,5-trinitrobenzene under the same conditions. If measured within an hour of mixing at room temperature, the two spectra, expressed in terms of the formal molar extinction coefficient (optical density/molarity of nitrocompound) do resemble one another closely. Both solutions obey Beer's law and both bear a close resemblance to Meisenheimer's compound in acetone[3]. After a day, the spectrum of 1,3,5-trinitrobenzene in excess potassium ethoxide resembles that obtained by Ainscough and Caldin. Similar results were obtained using sodium ethoxide. This suggests that 1,3,5-tri-

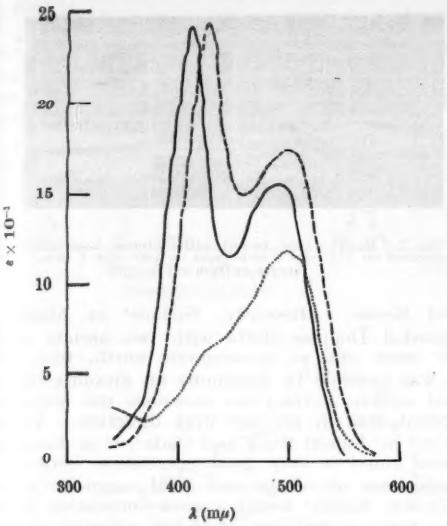


Fig. 1. ——, Potassium ethyl 1-methoxypicrate ($2{\cdot}27 \times 10^{-5}$ *M*) and potassium ethoxide ($6{\cdot}8 \times 10^{-5}$ *M*); – – – –, 1,3,5-trinitrobenzene ($3{\cdot}23 \times 10^{-5}$ *M*) and potassium ethoxide ($6{\cdot}8 \times 10^{-5}$ *M*) within 30 min. of mixing; . . . ., the same after 24 hr. All solutions in ethanol at 20° C.

nitrobenzene + ethoxide initially gives an addition product (I, R = H) which corresponds with the Meisenheimer compound.

The spectra were measured on an Optica *CF*4 grating spectrophotometer.

This work was carried out during the tenure of an Edward A. Deeds Fellowship of the University of St. Andrews.

R. Foster

Chemistry Department,
Queen's College, Dundee.

[1] Ainscough, J. B., and Caldin, E. F., *J. Chem. Soc.*, 2528 (1956).
[2] Meisenheimer, J., *Ann. der Chemie*, **323**, 205 (1902).
[3] Foster, R., *Nature*, **176**, 746 (1955).

Relation between the Polarizability of Ions and other Significant Properties

In a previous communication[1] it was reported that the polarizability α of an ion is proportional to N^2 (N being the atomic number of the element). In order to examine the extent to which this relation, $\alpha = kN^2$, where k is a constant, is applicable in the case of ions, the data for the polarizabilities of ions[2] were plotted against the values of N^2 for the corresponding ions. It was found that it is applicable for elements in the same sub-group of the periodic table, although the proportionality constant k in this relation for earlier members of each sub-group differs from that applicable to the higher members of the same sub-group.

By applying this criterion, the element fluorine is found to differ from the other halogens, chlorine, bromine and iodine, in having a lower polarizability than that to be expected from the above formula. Similarly, lithium and sodium ions differ from potassium, rubidium and cæsium ions in group 1*A*, beryllium and magnesium differ from calcium, strontium, and barium ions, and so on. Such differences in the behaviour of the earlier elements in the sub-groups from those occurring higher up are in agreement with the corresponding differences in the chemical behaviour between fluorine on one hand *and chlorine, bromine and iodine on the other*, lithium and sodium from potassium, rubidium and cæsium and so on. This is evidently to be attributed to the electronic configurations in the atoms.

It is found also that for elements belonging to the same series in the different periods, the values of α decrease with increase in N^2.

Another interesting correlation has been obtained by plotting the values of the electric polarizabilities of ions against their magnetic susceptibilities, χ (see ref. 2). The graph thus obtained shows a distinct proportionality between the two, the relation being $\alpha = K_0 \chi$, where K_0, the constant of proportionality, is different for positive and negative ions.

K. Krishnamurti

Physico-Chemical Laboratories,
College of Science,
University of Baghdad.

[1] Krishnamurti, K., *Nature*, **182**, 1227 (1958).
[2] Data taken mostly from Van Vleck's "Electric and Magnetic Susceptibilities", 225 (Oxf. Univ. Press).

PHYSICS

Nuclear Magnetic Resonance in Rolled Copper Sheet

An investigation has been made of the copper-63 nuclear magnetic resonance in rolled copper sheet. The specimens were made from an electrolytic copper of commercial purity and consisted of a pile of 18 sheets each measuring 0·6 cm. × 0·5 cm. × 0·0045 cm. and insulated from each other by rice paper of thickness 0·003 cm. The measurements were made with a Colpitts marginal oscillator at a frequency of 5·5 Mc./s., using a permanent magnet with pole pieces of 4·5 in. diameter and 1·5 in. gap.

The following features were observed.

(1) In sheet which has been recrystallized after heavy deformation by rolling (90 per cent reduction in thickness or more) the width of the line varies with the orientation of the specimen relative to the main magnetic field. This effect occurs for all annealing temperatures investigated (240–600° C.) but is more pronounced for the higher temperatures. If the specimen is rotated about the transverse direction (the direction in the plane of the sheet perpendicular to the rolling direction) the line-width shows minima for $\theta = 0$ and $\theta = \pi/2$, and a maximum for $\theta = \pi/4$, where θ is the angle between the main magnetic field and the plane of the sheet. The magnitude of the variation may be as much as $\pm$ 15 per cent in the mean square line-width.

This effect can be explained by the Van Vleck theory of dipolar broadening[1] if it is assumed that there is present in the specimen a preferred orientation of the type usually observed in recrystallized rolled sheet[2].

A variation of line-width with θ is also found for the deformed sheet; this is of a different nature from that in the annealed sheet, and is more difficult to interpret in terms of the expected preferred orientations.

(2) Annealing of a deformed specimen produces no detectable change in the shape of the resonance line, nor in its width except in the case of the most heavily deformed specimens (90 per cent reduction in thick-

ness). With these last specimens at all values of θ, except those near $\theta = \pi/4$, there is a narrowing of the line on annealing which is ascribed to a change in the preferred orientation; for $\theta = \pi/4$ there is no measurable change in the width. However, annealing causes an increase in the intensity of the line by about 25 per cent above that of the deformed sheet, and this increase appears to be independent of the degree of deformation in the range 25 per cent to 90 per cent reduction in thickness by rolling.

(3) For specimens of deformed sheet given a succession of heat treatments at temperatures increasing in steps of 10° C., the increase in intensity of the line is found to have taken place during treatments in a range of about 40° C., the variation being qualitatively similar to the variation in other properties of copper known to accompany recrystallization. The ranges of temperature are 200–240° C. for 90 per cent reduction and 230–270° C. for 50 per cent reduction. There is no apparent change in the shape of the line at any stage.

It has been reported by Bloembergen and Rowland[3] that the intensity of the absorption line in filed copper increases on annealing by a factor of up to 2, and this is ascribed to the non-appearance of the 'satellite' components of the line in the deformed material because of quadrupole interaction between the nucleus and the electric field gradients produced by dislocations. A similar explanation seems to be difficult to apply in the present case because of the absence of any marked broadening of the line: the observed increase would imply that in the deformed material about one-third of the nuclei are lying in a field gradient large enough to render the satellite components of their nuclear resonance unobservable, while the remaining two-thirds of the nuclei have their resonance virtually unaffected. Such an interpretation seems to imply either a discontinuous distribution of strain in the specimen, or a non-linear relation between strain and quadrupolar interaction energy. The discontinuities in strain which occur in the immediate neighbourhood of a dislocation line are not sufficient to explain the effect, since even if the dislocation density in the deformed material is as high as 10^{11} lines/cm.^{2}, only about 1 per cent of the atoms will lie within 5 interatomic distances of a dislocation line.

E. A. Faulkner

Division of Tribophysics,
Commonwealth Scientific and
Industrial Research Organization,
University of Melbourne. March 6.

[1] Van Vleck, J. H., *Phys. Rev.*, **74**, 1168 (1948).
[2] Barrett, C. S., "Structure of Metals" (McGraw-Hill, 1952).
[3] Bloembergen, N., and Rowland, T. J., *Acta Met.*, **1**, 731 (1953).

Movement of Auroral Echoes and the Magnetic Disturbance Current System

A number of workers have reported systematic movement of very high-frequency radar echoes associated with auroral ionization. Bullough and Kaiser[1] at Jodrell Bank in England interpreted their results as indicating a westward movement before 2100 hr. local time and eastward thereafter. Unwin[2] at Invercargill in New Zealand, with an aerial directed 20° east of geomagnetic south, recorded radial motions which were northward before about 2230 hr. local time on the average, and southward thereafter. These results are consistent with those of Bullough and Kaiser. Recently, Nichols[3] in Alaska has recorded Doppler shifts with two aerials directed 30° each side of geomagnetic north, from which it was possible to determine an absolute direction and velocity. On most occasions the major component was in an east–west direction. Bullough *et al.*[4] at Jodrell Bank and Gadsden[5] at Invercargill have found a very good correlation between the occurrence of echoes and local magnetic activity. Further, Kaiser[6] found a good correlation between the mean component of radial velocity of echoes (in a direction 50° west of geomagnetic north) and the mean vertical component of the magnetic disturbance vector at Eskdalemuir for the days on which echoes were observed at Jodrell Bank.

Fig. 1. Range–time record with antenna beam alternately oriented on 175° (for 6 min.) and on 240° (for 4 min.). Time increases from left to right

From this type of evidence it has been suggested by both Bullough *et al.*[4] and Nichols[3] that the movements observed are probably those of the electrons in the magnetic disturbance current system as deduced by Vestine *et al.*[7] from magnetic observations. Nichols calculated a current density of 10^3 amp./km. of latitude, which is of the same order as that deduced from magnetic observations in the auroral zone. The present communication gives further evidence that the movements of echoes can be identified with the disturbance current system, and shows that there is rough quantitative agreement between the two phenomena.

Since May 1958, the radar at Invercargill operating at 55 Mc./s. has been arranged so that the centre of the antenna beam is oriented alternately on geographical bearings of 175° (for 6 min.) and 240° (for 4 min.). The orientations have been chosen to avoid a region of low probability of echoes centred at 210° approximately[8]. An example of a range–time record obtained with this programme is shown in Fig. 1. Consistent radial velocities in each direction are apparent over a period of 20 min., and it is justifiable to calculate an absolute velocity and direction from the radial components read from each consecutive pair of records. In the preliminary calculations no corrections have been made for the fact that the geographical direction of the radar beam varies slightly with range owing to the curvature of the Earth. This change is quite negligible at a bearing of 175°. At 240° it amounts to 10° at 1,200 km., which is small compared with the radar beam-width of 25°.

A histogram of all absolute directions observed between May and August is shown in Fig. 2*a*. They have been calculated separately for the range-intervals 0–400 km., 400–800 km. and 800–1,200 km. from the radar, but there is no systematic change in direction with range. The bulk of the directions measured are close to local magnetic east–west. On any one occasion the change from westerly to easterly movement during the night is quite distinct, though it may

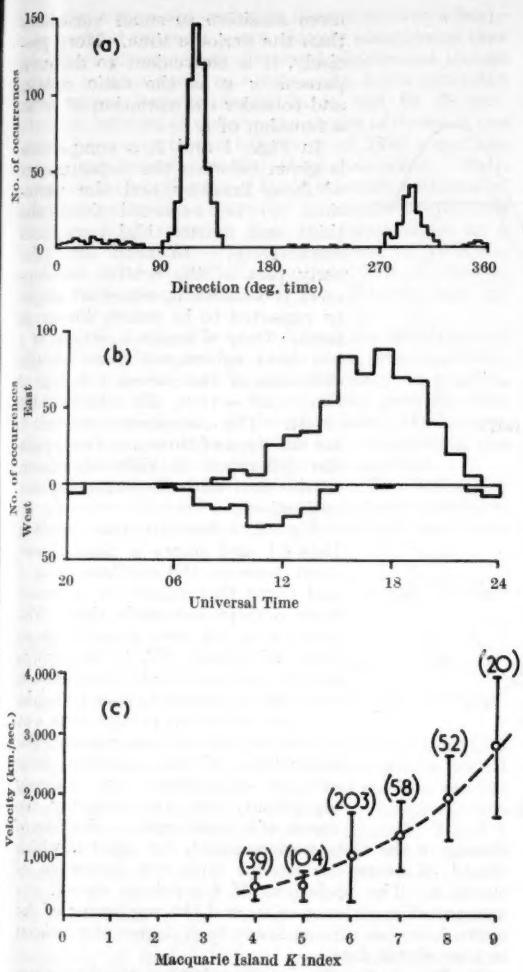


Fig. 2. Movement of echoes: (*a*) Number of occurrences vs. direction; (*b*) number of occurrences containing an easterly or westerly component in the direction vs. Universal Time; (*c*) Average velocities in the 800–1,200 km. range-interval vs. Macquarie Island *K* index. Limits are standard deviations in velocities and bracketed figures are the numbers of cases

occur at any time between about 1030 and 1400 U.T., as shown in Fig. 2*b*. The small number of points between 0 hr. and 0700 hr. U.T. is due to the fact that echoes are normally diffuse at this time[2] and do not reveal sufficient structure on a range–time display to derive an absolute velocity and direction by this method.

There is a wide scatter in the absolute velocities calculated, but in general they increase with the degree of magnetic activity. The average velocities of echoes in the range-interval 800–1,200 km. from the radar are shown plotted against Macquarie Island *K* index in Fig. 2*c*. Macquarie Island is 1,095 km. from radar on an azimuth of 214° geographic.

It has been shown[2] that the echoes come from a layer between a few kilometres and 25 km. thick centred at a height between 110 km. and a little more than 120 km. Recent rocket measurements by Meredith *et al.*[9] at Fort Churchill have revealed the existence of a current at a height of 120 km. during a magnetic disturbance. This evidence, combined with that from previous work discussed above, leaves little room for doubt that the observed movement of echoes can be identified with the magnetic disturbance current system. Following Chapman and Little's view[10] that the average electron density in the auroral zone is 3×10^5/cm.^{3}, a velocity of 1 km./sec. corresponds to a current of 720 amp./km. range for a layer 15 km. thick, assuming the ions are at rest. From Fig. 2*c* a velocity of 1 km./sec. is associated on the average with a *K*-index of 6 at Macquarie Island. Gadsden[5] has shown that for $K = 6$ the echoes extend to approximately 680 km. from the radar. If we assume further that the near boundary of the echo region corresponds to the boundary of the most intense portion of the disturbance current system, the magnitude of the disturbance vector at Macquarie Island may be found. An approximate calculation gives 375γ for the magnitude in this case, neglecting induced Earth currents. The departure from the quiet day curve for $K = 6$ at Macquarie Island is 360–600γ.

From this preliminary analysis it appears that the ionization giving rise to very high-frequency radar echoes occurs in the most intense portion of the disturbance current system, and that the observed movements of electrons can account for the magnitude of the disturbance vector. This conclusion gives some support to the work of Maeda[11], who found that, in order to interpret the diurnally varying part of magnetic storms by a dynamo theory, a strong polar wind system similar to that deduced here is required. More detailed observations are required to determine whether ions play a significant part.

The work, of which this is a preliminary report, forms part of the research programme of the Dominion Physical Laboratory of the New Zealand Department of Scientific and Industrial Research.

R. S. Unwin

Dominion Physical Laboratory Auroral Station,
Department of Scientific and Industrial Research,
Awarua Radio,
Invercargill, New Zealand.

[1] Bullough, K., and Kaiser, T. R., *J. Atmos. Terr. Phys.*, **6**, 198 (1955).
[2] Unwin, R. S., *Ann. Geophys.* (in the press).
[3] Nichols, B., *J. Atmos. Terr. Phys.*, **11**, 292 (1957).
[4] Bullough, K., Davidson, T. W., Kaiser, T. R., and Watkins, C. D., *J. Atmos. Terr. Phys.*, **11**, 237 (1957).
[5] Gadsden, M., *Ann. Geophys.* (in the press).
[6] Kaiser, T. R., "The Airglow and Aurorae", 156 (Pergamon Press, London and New York, 1955).
[7] Vestine, E. H., Laporte, L., Lance, I., and Scott, W. E., Pub. No. 580, 359 (Carnegie Inst., Washington, D.C., 1947).
[8] Unwin, R. S., *J. Geophys. Res.*, **63**, 501 (1958).
[9] Meredith, L. H., Davis, L. R., Heppner, J. P., and Berg, O. E., IGY World Data Centre A, Rocket Report Series No. 1, Nat. Acad. Sci., Washington, D.C., 169 (1958).
[10] Chapman, S., and Little, C. G., *J. Atmos. Terr. Phys.*, **10**, 20 (1957).
[11] Maeda, H., *J. Geomag. and Geoelect.*, **9**, 119 (1957).

Atmospheric Tides and Earth Satellite Observations

G. V. Groves, in a recent communication[1], offered an explanation of the marked change in slope of the periodic time curves of the satellites 1958 β, 1958 δ 1 and δ 2. His suggestion of a relatively sharp discontinuity in atmospheric density on passing from the dark to the light side of the Earth is, however, at variance with the work of Kelvin, Rayleigh and Margules on the ultimate effect of solar heating. From their investigations one would expect, as

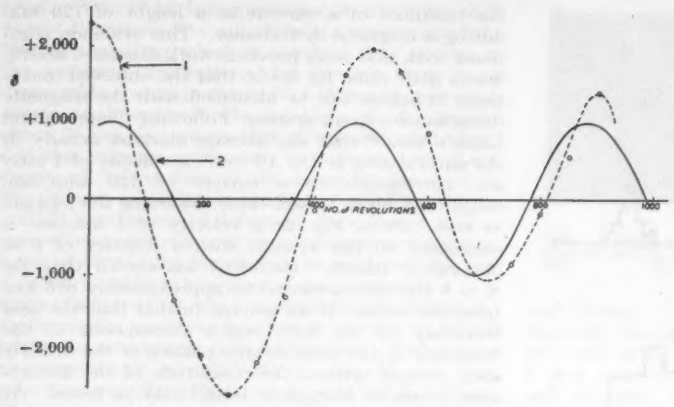


Fig. 1. Satellite 1958 δ 1 ; t_0 = 501·02 days after 1957·0

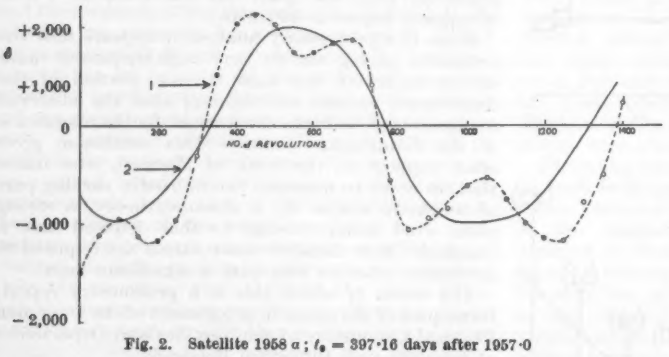


Fig. 2. Satellite 1958 α ; t_0 = 397·16 days after 1957·0

suggested by Kelvin, that the atmospheric distortion produced on the irradiated side of the Earth would lead to a range of Fourier components, that one being most easily detected which corresponded most closely with a natural frequency of tidal waves in the atmosphere. The marked semi-diurnal oscillation in barometric height is ascribed to this effect, and Margules[3] has shown that this corresponds to the natural frequency of waves in a rotating isothermal shell of temperature 298° K.

By a straightforward extension of Margules's analysis, one can investigate the possibility of standing waves of higher multiple frequencies. One finds resonance with the third harmonic in a shell of temperature 470° K., and with the fourth harmonic at a temperature of 590° K. This is relevant to the analysis of observation of satellites, since rocket probes and other sources[3] would suggest temperatures of this order at heights of between one and two hundred miles. More explicitly, one would expect a temperature of 470° K. at a height of about 130 miles, very near the level of the perigee of 1958 δ 1 at the beginning of its life.

In analysing observations of satellites, one has the choice of a variety of parameters, though the periodic time is usually chosen as being the most accurately measurable. Analysis of the dynamics of a satellite orbit under frictional drag, on the other hand, suggests as the most direct parameter the square of the product of the semi-major axis, a, and the eccentricity, e, since it is very nearly a linear function of the time, t. It has the further advantage of being more sensitive to small variations than the periodic time. More precisely, it is convenient to define a parameter u as the ratio ae/a_0e_0 and consider the variation of u^2 as a function of t.

In Figs. 1 and 2, a comparison is given between the departure of u^2 from linearity and the variations to be expected from the third and fourth tidal harmonics respectively. In each case the early part of the orbital development is considered, where u^2 might be expected to be purely linear in form. Only a linear function of t has been subtracted from u^2, the ordinates of the curves 1 being of form $[u^2 - (1 - Kt + C)] \times 10^4$ ($\equiv \delta$). The comparison curves 2 are the sines of three and four times the difference in right ascension of the Sun and the perigee point respectively.

Fig. 1 refers to the satellite 1958 δ 1 and shows a close agreement between the oscillations of δ and those that might be expected from a third harmonic tide. The sine curve has been given a phase shift of about 70°. One notes further that the oscillations show a decrease in amplitude such as might be expected as the perigee falls out of the region of resonance. The departures of the maxima from precise coincidence are scarcely significant, and are probably the result of a small error in the rate of change of the apex–perigee angle, the effect of which would of course be trebled with the formation of curve 2. The variations of δ nowhere exceed one quarter of a per cent of u, and the regularity of the curve 1 implies a remarkably high degree of precision in the orbital data.

In Fig. 2, for the satellite 1958 α, we find again a close agreement in form between δ and the sine curve, in this case the fourth harmonic with no shift in phase. Superimposed upon the main oscillation in δ is one of much smaller amplitude and higher frequency, which may be identified by its frequency alone as due to an equatorial bulge in the atmosphere of the type caused by the oblateness of the Earth. I have extended my analysis of this effect to meet the criticisms of King-Hele and Leslie[4], and find that this oscillation should have the form :

$$\left(\frac{K}{\omega}\right)\frac{\varepsilon R \sin^2\alpha}{2}\left(\frac{1}{H} - \frac{\beta 3}{a_0e_0}\right)\sin 2\beta$$

where K is the mean value of du^2/dt, ω the mean rate of increase of the apex–perigee angle β, ε and R the ellipticity and radius of the Earth respectively, α the inclination of the orbital plane to the equator and H the scale-height. The amplitude of this oscillation for 1958 α is of order 330×10^{-6}, agreeing well with the amplitude of the oscillations demonstrated.

It is difficult to avoid the conclusion that these harmonic tidal waves exist and indeed account for the major anomalies in the orbital observations.

Since the waves are probably operative over a fairly wide vertical range, it is not entirely coincidence that the effects of essentially localized phenomena should appear in two random orbits. As more extended observations become available, it will be of considerable interest to observe the region of decay of the fourth harmonic in the behaviour of 1958 α and the consequent growth of the third harmonic. Such observations would offer a fairly accurate estimate of temperature in the regions of maximum amplitude. Thus we might surmise that the temperature at a height of 224 miles—the perigee level of 1958 α—is of the order of 590° K., implying that the rise in temperature associated with the *F*-layer falls off above 200 miles.

While the major effects modifying the development of satellite orbits are probably now circumscribed, it is a matter of considerable intricacy to predict, for example, the precise values of the periodic time as the orbit develops. On the other hand, the 'young' orbit offers a convenient means for investigating the density distribution of the upper atmosphere.

I am indebted to the Smithsonian Institution for the observations used; without the extreme precision of these values, this discussion would not have been possible.

D. G. Parkyn

University of Cape Town.

[1] Groves, G. V., *Nature*, **182**, 1533 (1958).
[2] Margules, M., Smithsonian Misc. Collections, **843**, 296 (1893).
[3] Newell, H. E., *Ann. Geophys.*, **11**, 115 (1955).
[4] King-Hele, D. G., and Leslie, D. C. M., *Nature*, **182**, 788 (1958).

From the earliest data on the periods of satellites it was not clear whether the fluctuations in period were due to anything more than the random oscillations of an irregularly shaped body. Changes in the slope of the period–time curves of *Sputniks* 2 and 3 occurred over an interval of a few days, and I remarked that motion of the perigee due to the rotation of the orbital plane seemed to account for the suddenness of the change more easily than the slow drift of the perigee towards the equator. The frequency of these changes seemed related to the crossings of the perigee between the day and night sides of the Earth at three-monthly intervals, suggesting a diurnal variation in air density. As the analysis of data continued, more changes were evident and a periodicity of just less than one month has now been established, and this Parkyn associates with an 8-hr. density variation. The Smithsonian *Sputnik* 3 data, which he presents, show the variation in the period–time curve to be a very smooth oscillation in comparison with earlier data. This is evidence in favour of the phenomenon being a tidal oscillation, and also shows that the effect of the shape of the satellite is mainly averaged out.

One of the first results to be obtained from the launching of satellites was that the air density at 200 km. is several times greater than previously reckoned, due presumably to a higher temperature. Values for the scale-height at 200 km. which I have derived from satellite period–time curves are close to 40 km., and this value is supported by certain rocket measurements. The corresponding temperature is 1,100° K. on assuming the molecular weight for air to be 25 (or 700° K. if we suppose the nitrogen to be almost completely dissociated, which is unlikely). In either case, these values disagree with the values of 470° K. and 590° K., which Parkyn derives from the condition for the third and fourth harmonic oscillations.

G. V. Groves

Department of Physics,
University College, London.
March 12.

Time Relationship of Metre-Wave and 3-cm. Wave Bursts

It is known that solar bursts are observed over a very large range of wave-lengths. A single burst may extend over a large or small fraction of the range from centimetres to metres. Usually, bursts of strong intensity in the centimetre region are associated with great bursts observed on metre waves. It is generally believed that at the time of these bursts, radial ascension of ionized particles excites radio-frequency radiation at different levels in the solar atmosphere. Moreover, there are reasons for thinking that solar radio waves on the lower frequencies are emitted at a higher altitude in the solar atmosphere than those on the higher frequencies. As a result we observe a regular drift of the onset of the bursts towards the lower frequencies. The object of this communication is to discuss a relation existing between the times of commencement of the bursts observed on 3-cm. waves and on metre waves. This work is based chiefly upon measurements of the associated 3-cm. wave bursts made by means of an interferometer[1].

For this study, the data on metre-wave bursts (200 Mc./s.) provided by the observatory of Nera in Holland have been used. For those 3-cm. wave bursts which are accompanied by precursors, the time of beginning of the precursor has been taken as the time of beginning of the burst.

Fig. 1*a* shows the distribution of the times of commencement of the bursts observed on metre waves relative to those of the associated 3-cm. wave bursts. It will be seen that the metre-wave bursts often follow the associated 3-cm. wave bursts. Our results thus confirm that in the majority of cases the solar bursts are excited by the radial ascension of ionized particles in the solar atmosphere. It is also seen that the delay in time varies between zero and about 20 min. The average delay is about 3 min., corresponding to a velocity of radial movement of the order of 500 km./s.

In order to examine whether the delay in time depends on the dimensions of the bursts, the 3-cm. wave bursts associated with metre-wave bursts have been arbitrarily divided into two classes: (*a*) bursts having at the maximum intensity an apparent diameter less than 1·5 min. of arc on 3-cm. waves, and (*b*) the bursts having at the maximum intensity an apparent diameter greater than 1·5 min. of arc. Figs. 1*b* and 1*c* show the corresponding distributions of the delays of the metre-wave bursts relative to the 3-cm. wave bursts. It is seen that the delay has a tendency to be less for the bursts of smaller apparent diameter. The majority of cases in which the time-difference is small belong to class (*a*), whereas most of the cases observed where the time-difference is great belong to class (*b*). The average value of the delay for these two classes of bursts are of the order of 1 min. and 8 min. respectively. The corresponding velocities of the radial movement are of the order of 1,500 km./s. and 200 km./s. respectively.

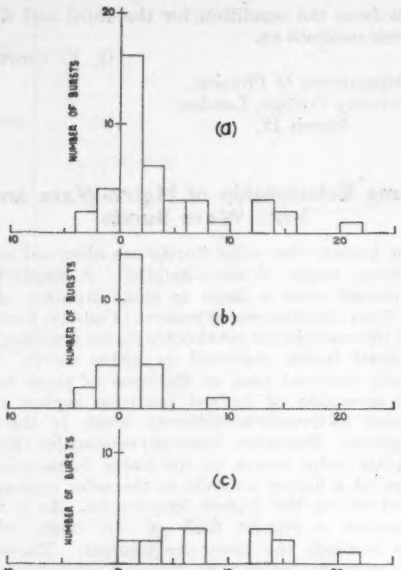


Fig. 1. Distribution of difference in time of commencement of metre-wave bursts relative to associated 3-cm. wave bursts: (*a*) all bursts, (*b*) bursts of diameter < 1·5 min. of arc on 3-cm., and (*c*) bursts of diameter >1·5 min. of arc on 3-cm. radiation

Typical bursts observed on 3-cm. wave-length, the equivalent temperatures of which can reach[2] 10^7 °K., are generally compared with bursts of types II and III usually observed on metre waves. However, it is difficult to identify without ambiguity these 3-cm. wave bursts with one or other of these types. In reality, the comparison between the 3-cm. wave bursts and those observed on longer wave-lengths shows that certain bursts occur almost simultaneously on all the frequencies and are consequently identifiable with bursts of type III. But on metre waves, the bursts of type III last only a few seconds, whereas the bursts observed on 3-cm. waves generally last several minutes. The longer duration of 3-cm. wave bursts agrees better with bursts of type II, which correspond to much greater time delays.

The statistical coincidence of bursts observed on 3-cm. waves and on metre waves shows that the two events have a tendency to occur almost simultaneously, that is to say, correspond to a rapid ejection (at a velocity greater than 1,000 km./s.) when the emissive region is of small diameter, whereas the delay in commencement of metre-wave bursts relative to 3-cm. wave bursts is greater (corresponding to velocities of the order of 500 km./s.) when the emissive region is of large diameter.

The work reported here was carried out at the Meudon Observatory under the guidance of Dr. J. F. Denisse, to whom I express my grateful thanks.

M. R. Kundu

National Physical Laboratory,
Hillside Road,
New Delhi, 12.
Feb. 19.

[1] Alon, I., Kundu, M. R., and Steinburg, J. L., *C.R. Acad. Sci., Paris*, **244**, 1726 (1957).
[2] Kundu, M. R., *C.R. Acad. Sci., Paris*, **246**, 2852 (1958).

Atmospheric Dust and Fluorescence

Many chemicals and minerals show a bluish fluorescence that can be destroyed by strong heating but is regenerated, after exposure to the air, by lesser heating[1]; I have called this reversibility of fluorescence by annealing. Stimulated by the well-known experiments of Ewles, I ascribed this to the action of adsorbed water, but recent experiments have shown that this interpretation is not sufficient and that the effect is due to dust most likely of organic origin deposited from the atmosphere; subsequent heating causes an interaction between this dust and the inorganic powder (SiO_2,Al_2O_3) used in these experiments, and this gives rise to stronger fluorescence.

The bluish fluorescence of 'pure' water[2] and of snow[3] is now also found to be due to such dust. For the fluorescence that can be destroyed by heating of magmatic minerals such as many feldspars that show no radiophotoluminescence, adsorbed water (Ewles effect) still seems the most plausible explanation; the presence in these minerals of traces of organic substances is, however, not impossible either, according to Oparin[4].

A more detailed report of this work will appear in the *Wiener Anzeiger*.

K. Przibram

Institut für Radiumforschung,
Vienna. Feb. 23.

[1] Przibram, K., *Nature*, **179**, 319, 864 (1957); **182**, 520 (1958).
[2] Carelli, A., Pringsheim, P., and Rosen, B., *Z. Phys.*, **51**, 511 (1928). Wawilow, S. I., and Tummermann, I. A., *Z. Phys.*, **54**, 270 (1929).
[3] Przibram, K., *Nature*, **182**, 520 (1958).
[4] Oparin, A. I., "Die Entstehung des Lebens auf der Erde" (3rd edit., Berlin, 1957).

The Three-dimensional or Solid-Image Microscope

In a recent communication[1] Gregory and Donaldson describe a prototype microscope which produces a three-dimensional image. In this instrument the microscope and the viewing screen oscillate relative to the object so that the microscope scans the object in depth and the viewing screen follows in phase the real image produced. The frequency of the motion is such that an apparent image of each successive plane is maintained in space without appreciable flicker because of the retention of the image by the eye. Reasonable success was claimed at low magnifications, × 20 being about the greatest useful magnification.

I have experimented with a somewhat simpler apparatus lacking the elegant device of the vibrating image screen. In this case the aim was to increase the depth of field at high magnifications by superimposing on the one virtual image plane, repetitive images of successive planes scanned in depth. This would, for example, allow the contents of a cell to be examined at the one focus under a magnification of × 1,000. Another important use for such an instrument would be the scanning of thick emulsions for particle tracks.

As with Gregory and Donaldson's instrument, the object is held to a sinusoidal oscillation along the optical axis by an alternating magnetic field. The period of the motion is 50 cycles/sec. using a tensioned diaphragm. This is powered by a hollow polarized solenoid surrounding the top of the condenser. The amplitude is controlled with a variable resistance in the low-voltage supply to the vibrator. With the present apparatus a depth of 20μ can be scanned without undue image distortion. However,

this is approaching the limit using oil immersion with a 50-cycle/sec. frequency. As Gregory and Donaldson mention, without correction the image in the central region of the scan would be weak, while that at the limits would be very bright by comparison. In the present apparatus the image brightness is rendered uniform by changing the phase so that the lamp brightness is proportional to the velocity of the scanning plane. By suitable adjustment of the impedance of the vibrator supply circuit, this supply is displaced some 80° out of phase with the supply to the microscope lamp. Thus, allowing for the lag in the filament temperature, the brightness increases with the velocity of the scan and falls to a minimum at the end of the stroke where the velocity is momentarily zero. Further critical control can then be exercised by varying the current to the lamp, thus altering the hysteresis in the filament.

The apparatus can be adjusted to give surprisingly good resolution at magnifications of × 1,000, using oil immersion objectives, provided that amplitudes less than about 15μ are used. No trouble was experienced with non-drying immersion oils of normal viscosity. The main difficulty at present is that standing waves and other imperfections leading to non-linear motion are present in the vibrator diaphragm. These may be corrected for any position; but readjustment is required with each new position of the slide. The inherent problem is to provide strictly linear motion without increasing bulk or momentum.

W. D. Jackson

Botany Department,
University of Tasmania,
Hobart. Jan. 28.

[1] Gregory, R. L., and Donaldson, P. E. K., *Nature*, **182**, 1434 (1958).

GEOPHYSICS and GEOCHEMISTRY

Remanent Magnetism of the Cretaceous Basalts of Madagascar

A general study of the remanent magnetism of volcanic rocks from Madagascar has been undertaken since the beginning of 1958 under the direction of Dr. H. Besairie, chief of the Geological Department of Madagascar.

From various places in the island, more than a hundred oriented samples have been collected by one of us (L. C.) and seven samples by J. Boulanger and P. Bussiere. Forty samples belong to flows or dykes well dated from the upper Cretaceous. Their ages have been proved to vary between Cenomanian and Santonian[1].

The samples were taken in places widely distributed along the eastern coastal district and the western part of the island. They were generally collected in quarries or trenches, some metres below ground-level, but sometimes at ground-level in river beds. The rocks are dolerites, basalts or sakalavites.

In some places, Ampijoroa, Amborompotsy, Antsalova (part), the directions, as well as the values of the intensities of magnetization, proved widely scattered. Values as high as 33×10^{-3} c.g.s. units/gm. were found. It is believed[2,3] that lightning currents were responsible for these magnetizations. For the other places the intensities as well as the directions of magnetizations showed good consistency.

For samples originated from the same flow or dyke, the differences between the individual values obtained and the mean value vary from 0 to 4 deg. for inclination and from 0 to 20 deg. for declination.

According to the places, the mean values found for the intensities of magnetization vary from $0{\cdot}8 \times 10^{-3}$ to 9×10^{-3} c.g.s. units/gm.

The magnetic stability of the samples was tested by E. Thellier's method[4], that is, turning over the sample in the Earth's magnetic field for one to several months and comparing the values obtained before and after turning over. Except for two samples, the magnetization showed no variation.

The nature of the magnetization was tested on some samples by successive heatings in a non-magnetic furnace. Only one Curie point was observed between 400° C. and 600° C. for each sample. The magnetization is thought to be thermoremanent[5] and acquired at the time of cooling of the rocks.

All the samples are magnetized according to the sense of the present geomagnetic field at Madagascar. The mean values of the directions of magnetization for the stable samples at each place are given in Table 1. The inclinations are termed negative when the extremity of the magnetization vector points above the horizon.

Table 1

	Inc.	Dec.
Northern districts		
Sambava	−49° 30′	15° E.
Antalaha	−55°	39° E.
Samanavaka	−48°	18° W.
Central districts		
Fenerive	−62°	19° E.
Andramy	−51°	7° E.
Andafiha	−57°	18° E.
Antsalova	−47°	4° E.
Southern districts		
Manakara	−70°	36° E.
Vineta	−53°	26° E.
Tongobory	−56°	15° E.

The inclinations are systematically steeper by 2–10 deg. than those of the present magnetic field for the places on the eastern coast (Sambava, Antalaha, Fenerive, Manakara), but generally lower for the western districts (the differences from the present magnetic field being less than 7 deg.). Notwithstanding a dispersion of some degrees, the magnetic inclination of the samples increases with increasing latitude.

On the whole there is not much difference between the distribution of the magnetic inclination of the samples and that of the present geomagnetic field in the island, as we noticed in our first studies.

The declinations of the samples are all easterly, except for one place (Samanavaka). They differ widely from those of the present geomagnetic field in the island (which vary from 7° W. to 19° W.), the mean difference being nearly 30 deg.

The calculation of the orientation of the dipole axis corresponding to the magnetization of the rocks gave the values for the co-ordinates of the north geomagnetic pole as shown in Table 2.

Table 2

	Lat.	Long.
Northern districts	70° N.	161° W.
Central districts	70° N.	165° W.
Southern districts	64° N.	177° W.
Mean	68° N.	168° W.

The place calculated is in the vicinity of the Behring Strait (Fig. 1). S. K. Runcorn[7] has given results he obtained from the Cretaceous Dakota Sandstones the magnetization of which could correspond to a north geomagnetic pole at latitude 77° N. longitude 127° E., the sense of magnetization being

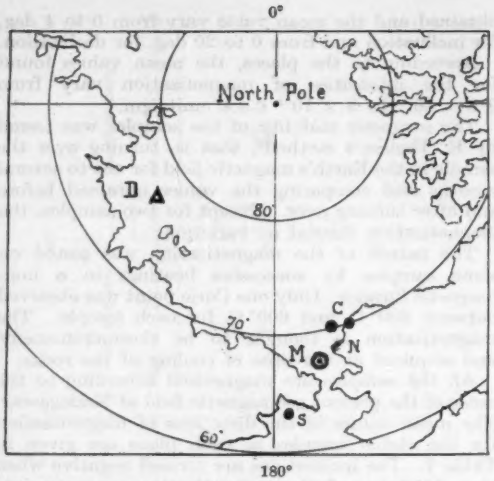


Fig. 1. North geomagnetic poles for Dakota (D, △); Madagascar, mean (M, ⊙); Madagascar, parts (N, C, S, ●)

reversed compared with that of the present terrestrial magnetic field.

The north geomagnetic pole for Madagascar is thus at a distance of less than 1,500 miles from the north geomagnetic American pole as given by Runcorn, and a little farther from the present geographical north pole.

This can be interpreted in different ways.

(1) The geomagnetic field, though having a dipole character for the main part, may have presented at times in the past a more or less complicated pattern, and the anomalies were smoothed out by taking the means of values scattered over long periods (of perhaps thousands or millions of years).

Therefore our results would have no significance in the perspective of polar wandering or continental drift.

(2) According to Runcorn's hypothesis[8], the mean geomagnetic field obtained by taking the mean of values scattered over a long period is a geocentric dipole field; our results would give the mean orientation of the dipole relative to Madagascar during a part of the upper Cretaceous.

(a) If the axis of the dipole had undergone, in the past, angular displacements of limited amplitude comparatively rapidly, say, for example, 10–20 deg. in one or a few million years, then the difference between our results and those of Runcorn could be most simply interpreted by the fact that the samples of America and of Madagascar do not belong exactly to the same period and the results give a picture of the orientation of the dipole at two different periods of the Cretaceous.

The opposed polarities of magnetization for the samples of Dakota and Madagascar give support to this hypothesis.

(b) If the dipole axis has undergone in the past displacements only at a slow rate (whether the dipole coincided or not with the axis of rotation of the Earth), we would have to think of relative continental drift for Madagascar and North America. From the Cretaceous up to the present time, according to calculation, the drift for Madagascar would have been about 1,500 miles in amplitude with a direction north-north-easterly.

Though displacements of much larger amplitude have been contemplated for Australia[9] and the Deccan peninsula[10], this hypothesis is considered by us as less likely than that of paragraph 2a above.

We wish to express our thanks to Prof. E. Thellier, whose advice was of inestimable value, and to Dr. H. Besairie, who has organized the sampling part of the work and provided the stratigraphical dating. We are also indebted to Prof. H. Dessens, director of the Puy de Dôme Observatory, and to Prof. M. Roques, who have greatly facilitated these studies.

A. Roche

Observatoire du Puy de Dôme,
Université de Clermont,
12 Avenue des Landais,
Clermont-Ferrand.

L. Cattala

Observatoire de Tananarive.

Feb. 10.

[1] Besairie, H., and Collignon, M., "Lexique internat. de Stratigraphie", 4, Afrique, 2, Madagascar, 24 (1956).
[2] Roche, A., thesis, Paris (1953).
[3] Hales, A. L., *J. Mon. Not. Roy. Astro. Soc.*, Geophys. Supp., 1, 3, 249 (1958).
[4] Thellier, E., *C.R. Acad. Sci., Paris*, **204**, 876 (1937).
[5] Thellier, E., *J. de Phys.*, **12**, 205 (1951).
[6] Roche, A., Cattala, L., and Boulanger, P., *C.R. Acad. Sci., Paris*, **246**, 2922 (1958).
[7] Runcorn, S. K., *Bull. Geol. Soc. Amer.*, **67**, 301 (1956).
[8] Creer, K. M., Irving, E., Runcorn, S. K., *Phil. Trans. Roy. Soc.*, A, **974**, 250, 144 (1957).
[9] Irving, E., *Mon. Not. Roy. Astro. Soc.*, Geophys. Supp., 1, 3, 224 (1958).
[10] Clegg, J. A., Radakrishnamurty, C., and Sahasrabudhe, P. W., *Nature*, **181**, 830 (1958).

Biological Origin of Manganese-rich Deposits of the Sea Floor

Recently, one of us has advanced arguments[1] in support of the idea that manganese-rich deposits of the sea floor may result from the metabolic activity of a bottom-dwelling organism that derives its nutrients from organic complexes in the water. We present here some new evidence that is relevant to that concept.

On December 18, 1958, at lat. 34° 48′ N., long. 60° 30′ W., in 5,139 m. of water, Mr. L. V. Worthington on *Chain* Cruise No. 2 retrieved about 50 gm. of light tan mud and preserved it for us in borate-buffered (*p*H 8) formalin. The present bottom temperature at this site is 2·3° C. The finer fractions of this material were removed by suspension in water and decantation, and it was possible to isolate a few mgm. of a relatively coarse fraction, which, for the most part, consists of the vacant tests of agglutinated arenaceous (? *Rhabdammina* sp.) and of calcareous Foraminifera. It is of particular interest that almost all the former are coated inside and out with a dark brown or black, earthy or shiny veneer which, by one-dimensional paper chromatography, is seen to be markedly similar in composition to manganese-rich nodules from the Blake plateau, that is, containing appreciable quantities of nickel, cobalt and copper as well as abundant manganese and iron. Treatment with 6 N hydrochloric acid removes the dark coating and leaves the Foraminifera tests intact, thus demonstrating that the manganese-rich material was formed as a surface deposit, and not through the capture of pre-existing manganese-rich particles by the living Foraminifera. All manganese-rich particles in this sample apparently were formed by deposition on agglutinated Foraminifera the natural surfaces of which seem to be studded with detrital quartz grains; no comparable manganese-rich deposits are visible

on calcareous tests or on isolated sand grains. That the uncoated agglutinated Foraminifera have on their surfaces some protein-rich material is suggested by their adsorption of rose bengal dye[3], and by their reaction with ninhydrin.

To us, these observations support the argument that manganese-rich deposits of the sea floor originate through biological rather than inorganic processes. It would seem that the protein-rich exudate of these agglutinated Foraminifera has provided a favourable substrate which promoted the growth of a colony of organisms that are capable of extracting trace elements from sea water. Siliceous detrital grains on the sea floor, that were lacking in the protein-rich coating, were not such biologically attractive sites, nor were the calcareous tests of other associated Foraminifera.

We are now making preparations for a careful search for the organism which, we expect, is responsible for this type of deposition of trace elements from sea water.

This work was supported, in part, by Contract *Nonr*-2196 with the Office of Naval Research, U.S. Navy.

JOHN W. GRAHAM
SUSAN C. COOPER

Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts.

Feb. 27.

[1] Graham, John W., *Science* (In the press).
[2] Walton, W. R., *Cushman Found. for Foram. Res.*, 3, 56 (1952).

BIOCHEMISTRY

Amino-Acetone: its Isolation and Role in Metabolism

A PRELIMINARY study[1] of the properties of a metabolite produced by washed suspensions of *Staphylococcus aureus* led to the provisional assignment of the structure $CH_3.CO.CH_2NH_2$. This compound, hitherto undetected in Nature, has now been isolated in crystalline form and positively identified as amino-acetone.

The present communication is concerned with the broad details of the isolation and characterization of amino-acetone and, in addition, presents a hypothesis which accounts for its formation and catabolism. It is suggested that this latter involves glyoxalase, an enzyme system which, as yet, has been without a definitive role in intermediary metabolism.

The accumulation of amino-acetone by *Staph. aureus* cells harvested from a bulk culture medium and suspended in phosphate buffer (*p*H 7·0) containing 0·02 *M* DL-threonine was measured at intervals using a modified picrate reaction method[2,3]. During a 20-hr. incubation a total of 15 m.moles of the compound was produced; it was isolated by adsorption and elution from 'Amberlite *IRC* 50', fractionation with phosphotungstic acid and finally by chromatography on paper. It was obtained as the pure compound by precipitation from ethanol, as the salt of *p*-toluene sulphonic acid. This salt was recrystallized twice from absolute ethanol–diethyl-ether as fine white needles—yield 0·39 gm. (m.p. 130·5°).

Elementary analysis gave the following results: amino-acetone *p*-toluene sulphonic acid salt; C, 49·2; H, 6·3; N, 5·9; S, 13·0. $C_{10}H_{15}O_4NS$ requires: C, 49·0; H, 6·2; N, 5·7; S, 13·1 per cent.

Amino-acetone hydrochloride was synthesized via acetonylphthalimide[4] and converted to the *p*-toluene–sulphonic acid salt. This was recrystallized twice from absolute ethanol–diethylether and yielded long thin leaflets with m.p. 130·5°. On analysis it gave the following data: C, 49·2. H, 6·4; N, 5·9; S, 12·9 per cent. A mixture of the synthetic material, and that isolated from the culture, also melted at 130·5°.

The behaviour of the natural and synthetic materials was identical when chromatographed on paper in a number of solvent systems and when subjected to paper electrophoresis. The identical nature of both products was also confirmed by infra-red spectroscopy, which was kindly carried out by Dr. E. Spinner.

It was shown[1] that a compound, now identified as amino-acetone, was formed by *Staph. aureus* either rapidly from threonine alone or slowly from glycine plus glucose, oxygen being necessary in both cases. The question now arises as to the mode of formation of amino-acetone from the two amino-acid precursors. In the case of glycine it would seem likely that it condenses with an acetyl derivative to give α-amino-β-ketobutyric acid in a manner analogous to the formation of α-amino-β-ketoadipic acid from a succinyl derivative and glycine, by the pathway proposed for the synthesis of δ-aminolævulinic acid[1,5–7]. Thus it is envisaged that acetyl-coenzyme *A*, derived from glucose, condenses with glycine as shown in equation 1. The α-amino-β-keto acid so formed would then give rise to amino-acetone by a simple decarboxylation (equation 3). In this connexion it is of interest to note that Gibson *et al.*[5] have independently reported the formation of an amino-ketone from acetyl-coenzyme *A* and glycine by red blood cell preparations. In the case of threonine, a dehydrogenation reaction would yield α-amino-β-ketobutyric acid (equation 2) from which amino-acetone would arise as before.

$$CH_3.CO.S—CoA + CH_2NH_2.COOH \rightarrow CH_3.CO.CHNH_2.COOH + CoA—SH \quad (1)$$

$$CH_3.CHOH.CHNH_2.COOH \xrightarrow{-2H} CH_3.CO.CHNH_2.COOH \quad (2)$$

$$CH_3.CO.CHNH_2.COOH \rightarrow CH_3.CO.CH_2NH_2 + CO_2 \quad (3)$$

Regardless, however, of the actual mechanisms involved in the formation of amino-acetone, the appearance of this compound does suggest a role in metabolism, especially as it is known that amino-acetone labelled with carbon-14 is oxidized to $^{14}CO_2$ in the rat[8]. It is suggested that the above facts may be integrated into a metabolic cycle (Fig. 1) which could have a widespread occurrence in animal and bacterial cells.

It is postulated that amino-acetone, formed by the routes described above, is deaminated either by oxidation or transamination to yield methylglyoxal. If formed, methylglyoxal would be rapidly converted to D-lactic acid by glyoxalase and thence to pyruvic acid by the D-lactate dehydrogenase system[9–11]. Both these enzyme systems have a wide distribution in bacteria and in animal tissues.

The postulated cycle provides a new route for the oxidation of threonine and glycine and, in the latter case, an alternative to the succinate–glycine cycle[7]. In addition, it provides an interesting feature, namely, a possible source of substrate, and indeed a purpose, for glyoxalase, an enzyme system the place of which in the metabolic picture has been a puzzle for many years.

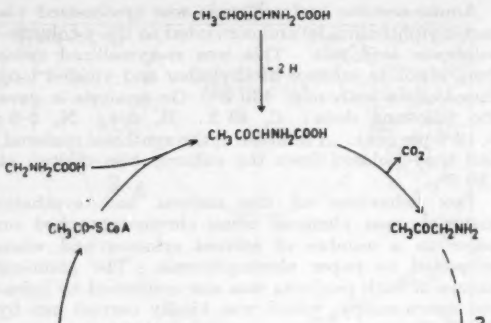


Fig. 1. Postulated amino-acetone cycle. The interrupted line represents a possible deamination reaction

The accumulation of amino-acetone in cultures of *Staph. aureus* is presumably due to the inability of this organism to deaminate the compound. Such a metabolic defect would be entirely in keeping with the limited nitrogen metabolism implied by the strict amino-acid requirements of *Staph. aureus*.

Experiments to test the validity of the above hypothesis are now in progress and, as with full details of the present work, will be reported later.

I wish to thank Prof. A. H. Ennor for the constant encouragement and advice which he has given throughout the course of this work.

W. H. Elliott

Department of Biochemistry,
John Curtin School of Medical Research,
Australian National University,
Canberra.

March 11.

[1] Elliott, W. H., *Biochim. Biophys. Acta*, **29**, 446 (1958).
[2] Shuster, L., *Biochem. J.*, **64**, 101 (1956).
[3] Elliott, W. H. (in preparation).
[4] Gabriel, S., and Colman, J., *Ber.*, **35**, 3805 (1902).
[5] Gibson, K. D., Laver, W. G., and Neuberger, A., *Biochem. J.*, **70**, 71 (1958).
[6] Kikuchi, G., Shemin, D., and Bachmann, B. J., *Biochim. Biophys. Acta*, **28**, 219 (1958).
[7] Shemin, D., Proc. Third Int. Congr. Biochemistry, Brussels, 197 (1955).
[8] Nemeth, A. M., Russel, C. S., and Shemin, D., *J. Biol. Chem.*, **229**, 415 (1957).
[9] Kaufman, S., Korkes, S., and Del Campillo, A., *J. Biol. Chem.*, **192**, 301 (1951).
[10] Huennekens, F. M., Mahler, H. R., and Fordmann, J., *Arch. Biochem.*, **30**, 77 (1951).
[11] Mahler, H. R., Tomisek, A., and Huennekens, F. M., *Exp. Cell Res.*, **4**, 208 (1953).

Identification of Barbiturates

In Great Britain the most common poisons at the present time are the 5 : 5′ disubstituted barbiturates. The longer-acting barbiturates, barbitone and phenobarbitone, can accumulate in the body when administered in relatively low therapeutic dosage, and concentrations of these drugs in the blood of about 5 mgm./100 ml. do not necessarily imply poisoning. The shorter-acting barbiturates, when taken in therapeutic quantities, however, do not accumulate, and in such cases this concentration would imply that an excessive quantity had been taken.

The only reliable method for the quantitative assay of barbiturate in 5–10 ml. samples of blood is ultra-violet spectrophotometry. The optical density difference at 240 mμ of aliquots at *p*H 10 and *p*H 2 is measured, and, because the E(1 per cent, 1 cm.) values for the various barbiturates are very similar, the total barbiturate concentration can be obtained. In order to assess the likely effect of this concentration it is necessary to identify the ingested barbiturate or at least assign it to the long- or short-acting group. Although slight differences in spectra between the various barbiturates can be detected and used for identification purposes[1], they are of value only if the background absorption is minimal and one barbiturate is present.

It is the purpose of this communication to direct attention to a test which has proved valuable in assisting in the identification of the various barbiturates. Maynert and Washburn[2] showed that concentrated sulphuric acid at room temperature dealkylated some secondary alkyl groups in the 5 : 5 disubstituted barbiturates, and Brooker[3], using treatment with acid at 100° for 1 hr., separated by paper chromatography the products from pentobarbitone and amylobarbitone. Combination of the techniques used by these workers has shown that if the products of the acid treatment at 100° for 1 hr. are, after dilution with water and extraction into ether, examined by ultra-violet spectrophotometry using 0·5 *N* aqueous ammonia as the solvent, then rapid and easy differentiation of several barbiturates is possible. The results of such treatment on the barbiturates commonly prescribed in the United Kingdom are shown in Table 1.

Table 1

	Result of treatment	Maximum of product in 0·5 *N* ammonium hydroxide
Barbitone Amylobarbitone (amytal) Butobarbitone (soneryl)	Unchanged	240 mμ
Pentobarbitone (Nembutal) Cyclobarbitone (phanodorm)	Attacked	268 mμ
Quinalbarbitone (seconal)	Attacked	Very weak absorption at 240, 266 and 315 mμ
Phenobarbitone	Completely destroyed	Nil

The products from the dealkylation of 'Tuinal' (mixed amylobarbitone and quinalbarbitone) give detectable absorption maxima at 240 mμ and 315 mμ. The absorption from the treatment of seconal is, however, very low and the 315 mμ peak is usually only observable when the extracts are reasonably pure.

The tungstic acid precipitation method followed by isolation of the weakly acidic ether-soluble fraction gives excellent products.

These techniques, coupled with those of paper chromatography[4], have, as yet, not failed to identify unambiguously any of the barbiturates involved in more than one hundred cases submitted to this laboratory in which therapeutic or toxic doses had been ingested.

A. S. Curry

Home Office Forensic Science Laboratory,
Rutland Drive,
Harrogate. Feb. 26.

[1] Maher and Puckett, *J. Lab. Clin. Med.*, **45**, 806 (1955).
[2] Maynert and Washburn, *J. Amer. Chem. Soc.*, **75**, 700 (1953).
[3] Brooker, *Analyst*, **82**, 448 (1957).
[4] Curry, *Acta Pharmacol. and Toxicol.*, **13**, 357 (1957).

Isomerization of Poly-*cis*-carotenes by Chlorophyll *in vivo* and *in vitro*

One of the *Chlorella* mutants described by Claes (strain 5/520)[1] synthesizes in the dark certain poly-*cis*-isomers, which are described in the literature as protetrahydrolycopene[2] and prolycopene[3]. In the algal cells the stereoconfiguration is sensitive to light. If oxygen is present, the procarotenes are isomerized in blue but not in red light, yielding the *trans*-forms in the cells. In this case light absorbed by the carotenes is presumed to be responsible for their isomerization.

Not so readily explained was the observation that in the mutant 5/520 the stereoconfiguration of protetrahydrolycopene and prolycopene can become unstable also in red light, which is not absorbed by carotenes. This phenomenon requires irradiation in a nitrogen atmosphere, absence of oxygen alone being insufficient[4].

In view of more recent results, the isomerization of protetrahydrolycopene and prolycopene under conditions of anaerobiosis and red light appears to be sensitized by the chlorophyll present in small amounts in the dark-grown mutant. After chromatographic isolation, both procarotenes show the same behaviour in petroleum ether as in the cell, if the solutions contain some chlorophyll.

Fig. 1 shows the chlorophyll-sensitized isomerization of protetrahydrolycopene in red light (500-W. tungsten lamp, Schott filter *RG* 1 and *KG* 1, distance of the solution from the light source about 60 cm.). Irradiation for 2 hr. results in a change from the spectrum of protetrahydrolycopene to one predominantly that of *trans*-tetrahydrolycopene. If the solution contains no chlorophyll, the procarotene spectrum is scarcely changed under the same conditions of irradiation.

As in the intact cells, chlorophyll-sensitized isomerization of poly-*cis*-carotenes in petroleum ether is inhibited by oxygen. Fig. 2 shows the influence of aeration on the isomerization of prolycopene. The carotene solution contained chlorophyll *a* and was irradiated with red light in the presence of nitrogen or air. Whereas the isomerization is almost at

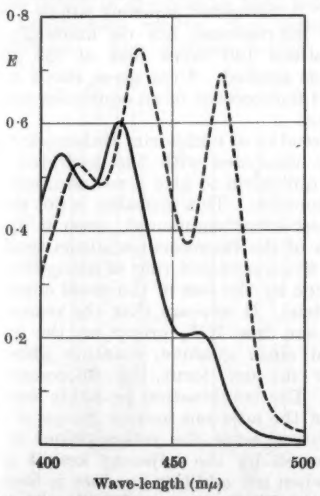


Fig. 1. Absorption spectrum of a mixture of protetrahydrolycopene ($6 \cdot 1 \times 10^{-5}$ *M*) and chlorophyll *a* ($0 \cdot 9 \times 10^{-5}$ *M*) in petroleum ether before, ——, and after, – – – –, irradiation for 2 hr. with red light

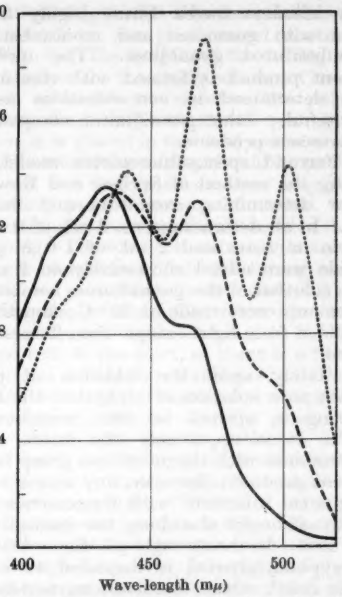


Fig. 2. Absorption spectrum of a mixture of prolycopene ($1 \cdot 2 \times 10^{-5}$ *M*) and chlorophyll *a* ($0 \cdot 2 \times 10^{-5}$ *M*) in petroleum ether before, ——, and after irradiation for half an hour with red light in the presence of nitrogen, ... , or air, – – – –

equilibrium after irradiation for half an hour in nitrogen, the presence of oxygen is inhibitory.

These findings indicate that in certain conditions (oxygen deficiency and light) the stereoconfiguration of carotenoids in the chloroplasts might be controlled by chlorophyll. The reaction is not limited to the structure of chloroplasts and can be observed outside the cell in pure solutions. Further implications necessarily await more detailed studies of chlorophyll – carotenoid interactions in general.

We are indebted to Miss I. Dobrigkeit for technical assistance.

H. Claes
T. O. M. Nakayama*
Max-Planck-Institut für Biologie,
Tübingen.

* Visiting research worker supported by the National Science Foundation.

[1] Claes, H., *Z. Naturforsch.*, **11***b*, 260 (1956); **12***b*, 401 (1957).
[2] Trombly, H. H., and Porter, J. W., *Arch. Biochem.*, **43**, 443 (1953).
[3] Zechmeister, L., Le Rosen, A., Schroeder, W. A., Polgar, A., and Pauling, L., *J. Amer. Chem. Soc.*, **65**, 1940 (1943).
[4] Claes, H., *Z. Naturforsch.*, **13***b*, 222 (1958).

Green Fluorescence of Guanidinium Compounds with Ninhydrin

Currently available methods for the determination of creatine and other guanidinium compounds are based on non-specific chromogenic reactions. These include the Jaffe, the diacetyl α-naphthol, the Sakaguchi, and the sodium nitroprusside–potassium ferrocyanide reactions. Determination of the individual members of the guanidinium group requires chromatographic separation, chemical degradation, or specific enzymatic destruction of the compound. In a search for a more satisfactory reaction for detection of guanidinium compounds than is at present afforded by chromogenic reactions we have found that ninhydrin (1,2,3-indantrione hydrate) in

strongly alkaline media forms highly fluorescent products with guanidine and monosubstituted or N,N-disubstituted guanidines. The most highly fluorescent product is formed with creatine, which can be determined in concentrations as low as 0·07 μgm./ml.; other guanidinium compounds form less fluorescent products.

The Farrand spectrofluorometer model *A*, calibrated by the method of Sprince and Rowley[1], was used for determining excitation and fluorescence spectra. In all determinations, 2 ml. of 0·5 per cent ninhydrin in water and 2 ml. of 1·0 *N* potassium hydroxide were added successively to 2 ml. of an aqueous solution of the guanidinium compound. All measurements were made at 24° C., and the sample was shielded from light except when fluorescence was measured.

Immediately upon the addition of potassium hydroxide to a solution of ninhydrin the five-membered ring is opened to form *o*-carboxyphenylglyoxal[2]. It is apparently the latter compound which combines with the guanidine group to give the fluorescent product; however, any excess *o*-carboxyphenylglyoxal interferes with fluorescence measurements by strongly absorbing the excitation wavelengths used. In the strongly alkaline solution excess *o*-carboxyphenylglyoxal is degraded to *o*-carboxymandelic acid[2], which is colourless, non-fluorescent, and has negligible absorbance at the excitation and fluorescence wave-lengths. Thus it is necessary to delay fluorimeter readings until this excess has been degraded and fluorescence reaches a maximum. With 0·5 per cent ninhydrin and 1·0 *N* potassium hydroxide a plateau of constant fluorescence occurs 9–14 min. after addition of alkali, during which time satisfactory fluorimeter readings may be made. Fluorescence decays to about half the initial value after 1 hr. and to 1/40 after 24 hr. Exposure to sunlight or to higher temperatures accelerates the rate of destruction of the fluorescent material.

The addition product of a guanidinium compound and ninhydrin is a highly fluorescent material with two sharp excitation peaks, at 305 and 390 mμ, the fluorescence at 390 mμ being approximately twice as intense as that at 305 mμ (Fig. 1). The fluorescence spectra have maxima at 495 mμ (Fig. 2). The spectra are similar for the guanidinium compounds tested; however, the amplitude of the peaks varies with the compound. It appears that N,N-disubstituted guanidines form the most highly fluorescent compounds, unsubstituted guanidine the least fluorescent, while monosubstituted guanidines are intermediate. The fluorescence of creatine, using an excitation wave-length of 390 mμ and measuring fluorescence at 495 mμ, is linear from 0·5 μmole to 75 μmole per litre.

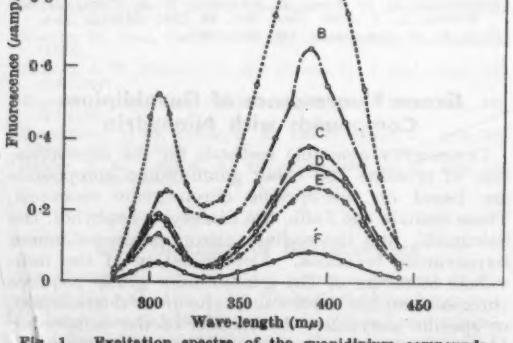


Fig. 1. Excitation spectra of the guanidinium compounds: *A*, creatine; *B*, dimethylguanidine; *C*, guanidoacetic acid; *D*, methylguanidine; *E*, arginine; *F*, guanidine. Each solution contains equal volumes of 0·5 per cent ninhydrin, 1·0 *N* potassium hydroxide and a 50-μmoles/l. solution of the guanidinium compound

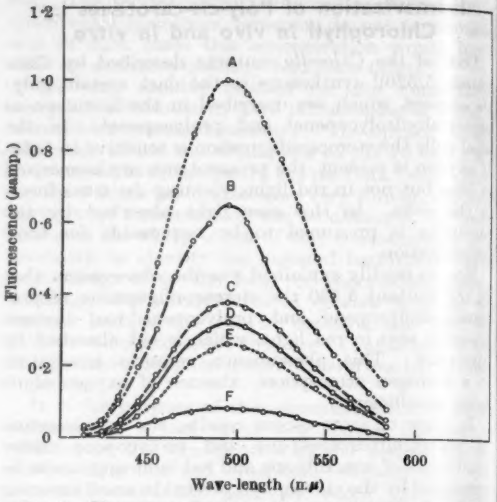


Fig. 2. Fluorescence spectra of the guanidinium compounds. Solutions are the same as for Fig. 1

Apart from the compounds the fluorescence spectra of which are presented in Figs. 1 and 2, creatine phosphate, creatinine, guanine and urea were studied. The fluorescence of creatine phosphate is less than 1 per cent, and the fluorescence of creatinine less than 0·5 per cent of that of an equimolar concentration of creatine. A saturated aqueous solution of guanine gives no fluorescence, nor do ammonium salts in concentrations 100 times that of the guanidinium compounds studied. Urea gives about 1/50,000 the calculated fluorescence of an equimolar concentration of creatine.

In his studies of ninhydrin, Ruhemann[2] noted that guanidine combines with the hydrated 2-carbonyl group of ninhydrin to give a red addition product in alkaline solution. This probably is not the structure of the fluorescent compound, since it appears that formation of the fluorescent addition product occurs after the five-membered ring of ninhydrin is opened, as indicated by the loss of the usual colour reactions of ninhydrin. It appears that the reaction requires at least one free NH_2 group on the guanidinium compound since guanine, creatine phosphate and creatinine do not form the fluorescent addition product. The combination probably occurs at one or both of the adjacent ketone groups of *o*-carboxyphenyl-glyoxal since the yellow colour of this compound caused by the adjacent ketone groups disappears when the addition product is formed.

Although the fluorescent reaction is non-specific among the guanidinium compounds, it provides a marked increase in sensitivity over the chromogenic

reactions currently used for the determination of these compounds. Application of the reaction to the determination of creatine and creatine phosphate in biological material is now being studied.

This work was supported in part by a training grant (*H.T.S.* 5222) given to the University of Minnesota by the National Heart Institute, United States Public Health Service.

REX B. CONN, JUN.
RICHARD B. DAVIS*

Clinical Laboratories and
Department of Medicine,
University of Minnesota,
Minneapolis. Jan. 15.

*Research Fellow in Medicine 1958–59, American College of Physicians.

[1] Sprince, H., and Rowley, G. R., *Science*, **125**, 25 (1957).
[2] Ruhemann, J., *J. Chem. Soc.*, **97**, 2025 (1910).

A Simple Method for the Concentration of Fluids containing Protein

THIS method is essentially based on the well-known principle of dialysis against substances of high molecular weight.

Fluid containing protein is placed in a glass tube or container, preferably with a small protuberance at the bottom end having approximately the smallest volume to which the fluid is to be concentrated. The size and shape of the glass container should be such as to provide the greatest possible dialysing surface. Using small glass tubes and narrow tubing, even a few millilitres of fluid containing protein can be successfully concentrated. The polyethylene glycol ('Carbowax' 20 *M*, made by Union Carbide, also available from G. T. Gurr), a waxy, flaky substance, with a molecular weight of 20,000, is broken up and an appropriate quantity of the dry granulated material is poured into the dialysis tubing. This can easily be achieved by inflating the tubing and using a funnel. 'Visking' dialysis tubing was used throughout, but any similar tubing would be suitable. The proportion of polyethylene glycol to fluid containing protein is not critical, but there should not be less than 1 part of polyethylene glycol to 10 parts of fluid. Polyvinylpyrrolidone (PVP) in dry powder form was also tried and gave satisfactory results; it is, however, much more expensive. The dialysis tubing containing the polyethylene glycol is then bent double (or more) and is placed in the glass vessel containing the glycol (Fig. 1). The length of the tubing should be such as to allow for the rising column of water inside it. It is advisable to moisten the polyethylene glycol inside the tubing with a small quantity of water just before it is placed in the fluid containing protein. The level to which the tubing should be pushed down depends on the final concentration required. The concentrating process ceases, of course, when the fluid containing protein reaches the level of the bottom of the tubing. The whole procedure is thus automatic. With larger quantities of fluid the tubing can be bent several times, providing a larger dialysing area and accelerating the process. The electrolyte content of the concentrate will be the same as in the fluid containing protein at the start, as there is a free passage of electrolytes across the membrane.

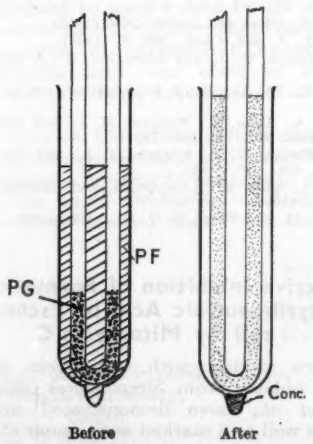


Fig. 1. Apparatus for concentrating fluids containing protein. *PF*, fluid containing protein; *PG*, polyethylene glycol; *Conc.*, concentrate

The method is rapid, reliable, and no special equipment is required. It has also the great advantage that the concentrate can be collected from clean glass and need not be scraped out from the inside of a sticky tube. Under suitable conditions a concentration, for example, of 10 ml. of urine to about 0·25 ml. is easily achieved in 3–4 hr. The high efficiency of the method is also due to the fact that the hydrophilic agent, namely, polyethylene glycol, does not contain water and has, therefore, full absorptive capacity. No denaturation effects were observed.

J. KOHN

Queen Mary's Hospital,
Roehampton, S.W.15.
Dec. 6.

Unfermentable Sugars in Barley Mash fermented by the Amylo Process

IN an earlier communication[1] concerning the production of alcohol from starchy materials by the amylo process, it was demonstrated that sugar consumption with barley fell considerably below that of the other raw materials by failing to exceed 85 per cent even with barley grains from which the bran had been removed. This was attributed to the presence of a considerable amount of unfermentable sugars. We now wish to report on the composition of residual unfermentable sugars in the fermented mash of barley, including the isolation of laminaribiose and of a β-linked glucosyl trisaccharide from this mash.

Polished barley grains were cooked with 0·03 per cent hydrochloric acid at 3 kgm./sq. cm. for 60 min. Alcohol fermentation of the cooked mash (total sugar 10·9 per cent) was carried out by the amylo process with the amylo mould (*Rhizopus javanicus* Takeda) and alcohol yeast (*Saccharomyces cerevisiae* sp.). After completion of fermentation, the mash (total residual sugar 1·6 per cent) was deproteinized and concentrated *in vacuo*. The acidic hydrolysis of this concentrate produced pentose (xylose and arabinose) and glucose as the sugar components detectable by paper chromatography; the pentose–glucose ratio of 44 : 56 was estimated on the basis of the paper chromatographic analysis devised by A. E. Flood *et al.*[2]. The concentrated sugar mixture (300 ml.) equivalent to 4,000 ml. of fermented mash was then separated into xylose, arabinose, galactose (?), three pentosyl oligosaccharides, five glucosyl oligosaccharides and higher pentosyl and glucosyl oligosaccharides by a combination of carbon column[3] and paper chromatographic techniques. Three pentosyl oligosaccharides had R_F

values of 0·29, 0·17 and 0·06 in *n*-butanol/pyridine/water (6 : 4 : 3), respectively. Further investigation on the structure of these pentosyl oligosaccharides was not carried out. Five glucosyl oligosaccharides consisted of two minor components presumed to be *iso*maltose (or gentiobiose) and kojibiose (or cellobiose), and three major constituents (*a*, *b* and *c*) having R_F values of 0·29, 0·16 and 0·05 in the same solvent, respectively. The ratio of *a*, *b* and *c* in the fermented mash was shown by paper chromatographic analysis to be 18 : 48 : 34. The greater part of each of these three sugars was eluted in the pure state (paper chromatography and ionophoresis[4]) from the carbon column; yields, 1·1 gm. of sugar *a*, 2·8 gm. of sugar *b* and 2·5 gm. of sugar *c*.

Sugar *a* was crystallized from methanol. The crystals were recrystallized from the same solvent: melting point 205–208°, unchanged on admixture with an authentic specimen of α-laminaribiose, $[\alpha]_D^{25} = +24{\cdot}8 \rightarrow +18{\cdot}8°$ (*c*, 2·5 in water). Acetylation of a portion of the free sugar yielded the crystals. The crystalline acetate was recrystallized from 95 per cent ethanol; melting point, 160·5–161·0°, undepressed on admixture with authentic β-octaacetate, $[\alpha]_D^{25} = -28{\cdot}8°$ (*c*, 2·5 in chloroform).

Sugar *b* was reducing and showed $[\alpha]_D^{25} = +10°$ (equil.: *c*, 3·0 in water). Acidic hydrolysis of sugar *b* gave only glucose on the paper chromatogram (identified as β-pentaacetate). From R_F and the elution behaviour from the carbon column, this substance was presumed to be a trisaccharide. A further indication was that the degree of polymerization calculated from the increase of reducing power[5] after hydrolysis was 2·8. The substance (1 gm.) was submitted to partial acidic hydrolysis with 1·0 per cent sulphuric acid at 100° for 2½ hr. Carbon column chromatography of the solution produced the mixture of disaccharides together with glucose and parent sugar. This mixture was fractionated by chromatography on paper into two components; the faster-moving component (0·11 gm.) and slower-moving component (0·1 gm.). Both were characterized by conversion into β-laminaribiose octaacetate and β-cellobiose octaacetate, respectively. This result showed that sugar *b* contained laminaribiose and cellobiose moieties. The sequence of linkages was determined by sodium borohydride reduction of sugar *b* to the sugar alcohol and partial acidic hydrolysis of the product. Paper chromatographic analysis revealed that the only reducing disaccharide present was cellobiose, thus proving that sugar *b* was O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)-D-glucose. It also supported the above structure that laminaribiose was the only disaccharide produced by the partial hydrolysis of β-glucosidase (almond emulsin)[6].

Acetylation was carried out with sodium acetate and acetic anhydride at 125–130° for 40 min. Crystalline β-hendecaacetate was formed from 95 per cent ethanol: melting point, 182·5–183·0°, $[\alpha]_D^{25} = -21{\cdot}7°$ (*c*, 3·0 in chloroform); analytical calculation for $C_{40}H_{54}O_{27}$: C 49·69, H 5·63 per cent; found: C 49·65, H 5·86 per cent. Adding the molecular rotation of laminaribiose and the molecular rotational increment in the series of cellodextrins gives +12·1°, which is in good agreement with $[\alpha]_D = +10°$ of our trisaccharide. Prof. S. Peat and his co-workers[8], however, reported 3-O-β-cellobiosylglucose (β-acetate: melting point, 108–110°, $[\alpha]_D = -8{\cdot}3°$) in the partial acidic hydrolysate of lichenin.

Acidic hydrolysis of sugar *c* also gave only glucose. It was shown to be a tetrasaccharide by the method described above. After partial acidic hydrolysis laminaribiose, cellobiose and trisaccharide mixture were detected, in addition to glucose and parent sugar. Consequently, it was inferred that sugar *c* was a tetrasaccharide containing at least one β-1 : 3- and one β-1 : 4-linkage.

The amylo process with starch materials other than barley showed 94–96 per cent sugar consumption and did not leave the above β-linked glucosyl oligosaccharides in such amount as is detectable on paper chromatogram. Further, the amount of these oligosaccharides isolated from the fermented barley mash was sufficiently large to preclude the possibility of their being products of transglucosidation by amylo moulds, since their power of transglucosidation is known to be extremely weak[7]. Furthermore, the main products of acid reversion of glucose include 1 : 6-linked disaccharides[8] and lævoglucosan[9]. In the actual experiment, no lævoglucosan was detected and only infinitesimal amounts of 1 : 6-linked sugars (*iso*maltose or gentiobiose) were isolated. Recently, R. A. Aitken *et al.*[10] detected laminaribiose and cellobiose in the enzymatic hydrolysate of barley glucosan. G. C. Aspinall and R. G. J. Telfer[11], who also studied the methylation of barley glucosans, indicated the presence of a polysaccharide containing unbranched chains of β-D-glucopyranose residues with approximately equal proportions of 1 : 3- and 1 : 4-linkages. From these facts, our β-linked glucosyl oligosaccharides are thought to be derived from the fragmentation of a β-glucosan.

We wish to thank Prof. T. Asai, Prof. K. Aso, Dr. K. Shibazaki and Mr. K. Mutai, president of the Fermentation Research Institute, for their helpful advice and interest in this work.

Hideo Ono
Michio Dazai

Fermentation Research Institute,
Agency of Industrial Science
and Technology,
Chiba, Japan.

[1] Ono, H., and Fukuoka, S., *J. Fermentation Assoc. (Japan)*, **10**, 413 (1952).
[2] Flood, A. E., Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, 1679 (1943).
[3] Whistler, R. L., and Durso, D. F., *J. Amer. Chem. Soc.*, **72**, 677 (1950).
[4] Foster, A. B., *Chem. and Indust.*, 1050 (1952).
[5] Somogyi, M., *J. Biol. Chem.*, **160**, 61 (1945).
[6] Peat, S., Whelan, W. J., and Roberts, J. G., *J. Chem. Soc.*, 3916 (1957).
[7] Shibazaki, K., and Aso, K., *J. Fermentation Tech. (Japan)*, **31**, 311 (1953).
[8] Thompson, A., Anno, K., Wolfrom, M. L., and Inatome, M., *J. Amer. Chem. Soc.*, **76**, 1309 (1954).
[9] Peat, S., Whelan, W. J., Edwards, T. E., and Owen (Mrs.), O., *J. Chem. Soc.*, 586 (1958).
[10] Aitken, R. A., Eddy, B. P., Ingram, M., and Weurman, C., *Biochem. J.*, **64**, 63 (1956).
[11] Aspinall, G. O., and Telfer, R. G. J., *J. Chem. Soc.*, 3519 (1954).

Selective Inhibition of Formation of Deoxyribonucleic Acid in *Escherichia coli* by Mitomycin C

Previous studies with mitomycin *C*, a new antibiotic isolated from *Streptomyces caespitosus* by Wakaki *et al.*, have demonstrated antibacterial activity as well as a marked antitumour effect[1,2]. In the course of investigating the mechanism of the actions of mitomycin *C*, evidence was obtained that

this antibiotic selectively inhibits the synthesis of deoxyribonucleic acid in *Escherichia coli B*.

Cells grown in a salts–glucose synthetic medium were harvested during the logarithmic phase, washed and resuspended in a similar fresh medium with or without mitomycin *C*, to give a concentration of 1×10^8 cells per ml. 20 ml. of the bacterial suspension were incubated at 35° with shaking, and aliquots of 2 ml. were taken at the intervals shown, and analysed for turbidity, nucleic acid and protein. Turbidity was measured photometrically at 660 mμ and viability of the cells was measured by plating. Ribonucleic and deoxyribonucleic acid were assayed by the orcinol and diphenylamine methods respectively, after extraction from the acid-insoluble precipitates of bacteria with hot 6 per cent perchloric acid. The protein content was measured by the Folin method.

Table 1. Effect of Mitomycin *C* on the Growth, Protein and Nucleic Acid Synthesis of *E. coli* B

Mitomycin *C* in suspension (μgm./ml.)	Time incubated (min.)	Turbidity E_{660}	Protein (Folin reaction) E_{660}	Ribonucleic acid (orcinol reaction) E_{660}	Deoxyribonucleic acid (diphenylamine reaction) E_{600}
0	0	0·090	0·070	0·110	0·030
	30	0·125	0·105	0·160	0·045
	60	0·180	0·150	0·225	0·065
	90	0·265	0·210	0·315	0·090
0·1	0	0·090	0·065	0·110	0·030
	30	0·135	0·100	0·155	0·030
	60	0·185	0·140	0·230	0·030
	90	0·225	0·190	0·320	0·030

Alterations in the bacteria during exposure to 0·1 μgm./ml. of mitomycin *C* are illustrated in Table 1. A normal increase in turbidity was observed during the first 60 min., after which the rate of increase in turbidity slowed down. However, after 90 min. of incubation there were only 1×10^6 viable cells/ml. as measured by plate count. Deoxyribonucleic acid formation was completely inhibited by the presence of mitomycin *C*, although synthesis of protein and ribonucleic acid was unaffected as compared with that of an unexposed culture during the test period. The inhibition of synthesis of deoxyribonucleic acid in the presence of the antibiotic could be reduced to some extent by the addition of baker's yeast extract to the incubation medium. The inhibitory action of mitomycin *C* on synthesis of deoxyribonucleic acid was observed even after removing the cells from mitomycin *C* and washing with saline.

Experiments on the effects of this compound on synthesis of deoxyribonucleic acid with the purified Kornberg enzyme are now in progress in an attempt to clarify the exact mechanism of the inhibitory action. A more detailed report will be published elsewhere.

S. Shiba
A. Terawaki
T. Taguchi
J. Kawamata

Research Institute for Microbial Diseases,
Osaka University,
Osaka.
Jan. 17.

[1] Wakaki, S., *et al.*, *Antib. and Chemo.*, **8**, 228 (1958).
[2] Hata, T., *et al.*, *J. Antib.*, Ser. A, **9**, 141 (1956).

ANIMAL PHYSIOLOGY

Biosynthesis of Ovalbumin

The problem of the biosynthesis of ovalbumin is interesting since this protein seems to be synthesized from a non-homogeneous pool of intermediary peptides[1–3]. We have studied the biosynthesis of this protein *in vivo* by the laying hen using L-cystine-^{35}S and $Na_2{}^{14}CO_3$. Selected birds were given these labelled substances and blood was obtained at stated intervals, either by heart puncture or from the wing vein. The time of laying the egg was carefully noted and ovalbumin was isolated from the egg white by ammonium sulphate fractionation. Serum globulin was isolated by precipitation with an equal volume of ammonium sulphate, while the total proteins were obtained by precipitation with trichloracetic acid to a final concentration of 5 per cent. In both cases the precipitates were repeatedly washed with 5 per cent trichloracetic acid to remove any co-precipitated amino-acids.

The results shown in Tables 1 and 2 indicate that the ovalbumin of eggs laid within 24 hr. of administration of the isotope was not significantly radioactive. The radioactivity of the ovalbumin is not high when that of the serum amino-acids is high. But the ovalbumin from eggs laid after 30 hr., when the radioactivity is found in the serum proteins and not in the serum amino-acids, is markedly radioactive. This shows that ovalbumin is not synthesized by the oviduct from the serum amino-acid pool, but probably from derivatives of serum proteins or intermediary peptide pools. The mechanism of the biosynthesis of ovalbumin is thus different from that of milk proteins, which have been shown to be derived from serum amino-acids[4].

Table 1. Incorporation of Carbon-14 Dioxide into Serum and Egg Protein

Bird No.	Time from administration of $^{14}CO_2$	C.p.m./ml. of serum, non-protein part	C.p.m./mgm. total serum proteins	Time of laying the egg from time isotope was given (hr.)	C.p.m./mgm. of ovalbumin isolated
1	17	2,000	19·8	44	104
	25	800	30·6		
	45	600	29·0		
2	8	3,300	237	22	nil
	25	1,930	230		
	45	400	266		

It could be argued that the oviduct might store serum amino-acids as a reserve and utilize them after 24 hr. That such storage of free amino-acids is not the case seems to be indicated by the following facts: (1) boiled juice from the oviduct has a very small content of free amino-acids as shown by the ninhydrin test; and (2) ovalbumin from eggs obtained as much as 48 hr. after the administration of the isotope is still radioactive. By this time the serum amino-acids have lost a considerable part of their radioactivity. It is difficult to appreciate that the amino-acids stored in the oviduct are not in equilibrium (even with a low exchange) with those in the serum.

However, such storage of a polypeptide precursor in the oviduct is not improbable, because our *in vitro* studies (Table 3) seem to indicate that the synthesis of ovalbumin by washed oviduct preparations is fairly independent of added amino-acids, adenosine triphosphate, etc. The amount of ovalbumin syn-

Table 2. Incorporation of Cystine-^{35}S into Proteins

Bird No.	Hr. after administration of cystine-^{35}S	Non-protein radioactivity c.p.m./ml. serum	C.p.m./mgm. of total serum protein	C.p.m./mgm. of globulin fraction	Time of laying egg from time isotope was given (hr.)	C.p.m./mgm. ovalbumin isotope	C.p.m./mgm. yolk protein
3	3	800		29·4			
	20	350	25·4	24·3	22	1·0	Nil
	49	Nil		52·0	49*	79·9	3·8
4	3	1,600	56·6	36·9			
	20	800	33·3	42·9	35	52·6	8·0
	49	Nil	35·7	34·1	49*	38·7	9·6

* The egg was obtained from the oviduct when the hens were killed.

Table 3. *In vitro* Synthesis of Ovalbumin by Oviduct Slices

Time of incubation (hr.)	Ovalbumin synthesized (μgm. per ml. of incubate)		
	Slices alone	Slices + amino-acids + ATP + FDP	Slices + amino-acids + ATP + FDP + hydroxylamine
1½	308	268	268
3	408	418	348
5	504	418	348

ATP, Adenosine triphosphate; FDP, fructose-1,6-diphosphate.

thesized in the *in vitro* incubation of oviduct with a complete medium, or in the absence of any additions, was estimated by antigen analysis techniques using rabbit anti-hen egg albumin sera. These sera were standardized previously with pure ovalbumin. These results show that the oviduct can synthesize ovalbumin without any further additions, and also that hydroxylamine does not have strong inhibitory action on this synthesis. This may possibly suggest a conversion of an ovalbumin precursor to ovalbumin not dependent on added adenosine triphosphate.

We wish to thank the National Institutes of Health, Bethesda, Maryland, for a grant in aid of this research.

D. V. Siva Sankar*
Henry W. Theis

Department of Chemistry,
Adelphi College,
Garden City, New York.

* Present address: Biochemical Research Laboratory, Creedmoor State Hospital, Jamaica 27, New York.

[1] Anfinsen, C. B., and Steinberg, D., *J. Biol. Chem.*, **189**, 739 (1951).
[2] Steinberg, D., and Anfinsen, C. B., *J. Biol. Chem.*, **199**, 925 (1952).
[3] Hendler, R. W., *J. Biol. Chem.*, **223**, 831 (1956).
[4] Askonas, B. A., Campbell, P. N., Godin, C., and Work, T. S., *Biochem. J.*, **61**, 105 (1955).

Molecular Orientation at the Surface of *Amoebae* during Pinocytosis

Pinocytosis[1] or cell-drinking appears to be a method for the uptake of molecules to which the cell membrane is impermeable[2]. In *Amoebae* the phenomenon may be identified by the formation of many short pseudopods each containing a central channel 1μ or more in diameter; droplets of fluid pinch off from this channel and migrate into the interior of the cell[3]. Recently, pinocytosis has become the subject of considerable physiological experimentation[2,4–6]. By means of fluorescein-labelled proteins, Brandt[5] demonstrated that proteins inducing pinocytosis are attached to the cell membrane of *Amoebae*. Schumaker[6] obtained results on the rate of uptake of radioactive proteins which lead to a similar conclusion.

In order to speculate intelligently about the induction of pinocytosis it would be of interest to know more about this attachment. Some information can be obtained from polarization microscopy, which will reveal the molecular orientation of the cell membrane and, in certain instances, of substances attached to the surface. The present preliminary communication describes interactions between pinocytosis-inducers and the surface of *Amoeba proteus* determined by the use of the polarizing microscope.

Mitchison[7] confirmed the well-known natural surface birefringence of *Amoebae* and showed that various basic dyes are oriented at the surface in an orderly molecular array *in vivo*. The surface orientation of these anomalously birefringent basic dyes may be shown by the striking differences in colour (for example, blue versus yellow) that appear when the surface faces different quadrants of the compensated polarizing microscope.

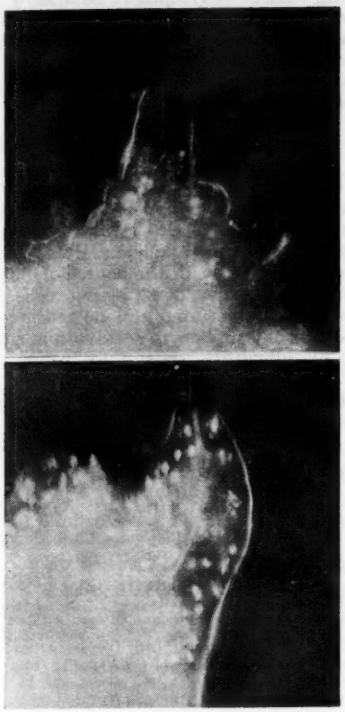
The first experiments were simple comparisons of cells in their inorganic culture medium and cells pinocytosing in either albumin or ribonuclease. There were no qualitative differences in the surface birefringence of the cells in the different media. The surfaces of the pinocytosis channels also showed the same molecular orientation as the rest of the cell surface (Fig. 1*a* and *b*). Hence, it may be concluded that there is no major re-arrangement of the orientation of molecules in the cell membrane. Quantitative studies of possible small differences do not appear to be feasible, because the errors in measurement[8] would vary as the square of the inaccuracy in determining the diameters of both the narrow channel and the wider pseudopod.

In the course of repeating Mitchison's experiments on the surface orientation of anomolously birefringent basic dyes[7], it was found that at higher concentrations toluidine blue not only became attached to the surface of *Amoebae* in a definite molecular orientation but also induced pinocytosis. There were no qualitative differences in dye orientation between the channels and other parts of the cell.

Two types of qualitative changes that have been described previously by Mitchison were seen in random parts of some cells. The first was the loss of dye-binding capacity but not natural birefringence accompanying surface 'bubbling'. The second was a 90° change in the orientation of the dye molecules with respect to the visible edge of the cell as evidenced by a colour change.

When the cells were first placed in dye solutions there were often strong differences in colour intensity between different parts of the surface of the same cell. Sometimes these differences could be discerned as absorption differences with transmitted (unpolarized) light. The increase in intensity could be watched on parts of cells which did not change shape. These observations indicate both local surface differences in the number of available dye-binding sites and a time-dependent attachment.

In order to test the hypothesis that proteins might also be absorbed to the same specific surface groups



a

b

Fig. 1

during the induction of pinocytosis, a series of experiments was performed using both proteins and toluidine blue. Three designs were employed: simple competition with both pinocytosis-inducers present, addition of the dye to a protein solution containing pinocytosing *Amoebae*, and addition of protein to a dye solution containing pinocytosing cells. Four proteins were tested separately: albumin, protamine, ribonuclease, and trypsin. In all instances there was competition dependent on concentration between the dye and the protein for surface sites as evidenced by the comparison of natural surface birefringence with the anomalous birefringence colours of the attached dye. Ribonuclease and protamine, two basic proteins, appeared to be more effective than either albumin or trypsin; however, the relative effectiveness will require further examination under conditions of varying *p*H and salt concentration.

The competition experiments suggest that proteins are not only attached to the cell surface[5,6] but also that they are attached in an orderly molecular array. Since only basic dyes are oriented at the cell surface[7] and the basic proteins appear to compete most favourably with these dyes, it is also reasonable to postulate that the sites on the cell surface are acidic.

This method, while indirect, is highly sensitive, and should prove to be a valuable tool for the further study of surface interactions in the induction and inhibition of pinocytosis.

This work was performed during the tenure of a research fellowship of the National Cancer Institute of the U.S. Public Health Service. I wish to thank Dr. J. M. Mitchison not only for instruction in the techniques of precision polarization microscopy but also for suggesting re-examination of the dye orientation. I am also grateful to Dr. Mitchison, Prof. M. M. Swann, Dr. N. de Terra, and Prof. H. Holter for stimulating discussions about these problems.

RONALD C. RUSTAD*

Department of Zoology,
University of Edinburgh.
Jan. 23.

* Present address: Department of Biological Sciences, Florida State University, Tallahassee, Florida.

[1] Lewis, W. H., *Johns Hopkins Hosp. Bull.*, **49**, 17 (1931).
[2] Chapman-Andresen, C., and Holter, H., *Exp. Cell Res.*, Supp., **3**, 52 (1955).
[3] Mast, S. O., and Doyle, W. L., *Protoplasma*, **20**, 555 (1934).
[4] Holter, H., and Marshall, J. M., *C.R. Lab. Carlsberg*, Ser. Chim., **29**, 7 (1954). Chapman-Andresen, C., and Prescott, D. M., *ibid.*, **30**, 57 (1956). de Terra, N., and Rustad, R. C., *Exp. Cell Res.* (in the press). Holter, H., *Internat. Rev. Cytol.* (in the press).
[5] Brandt, P. W., *Exp. Cell Res.*, **15**, 300 (1958).
[6] Schumaker, V. N., *Exp. Cell Res.*, **15**, 314 (1958).
[7] Mitchison, J. M., *Nature*, **166**, 313 (1950).
[8] Mitchison, J. M., *J. Exp. Biol.*, **39**, 397 (1952).

Re-utilization of Lymphocyte Remnants by Reticular Cells

THE behaviour and function of lymphocytes were discussed at a meeting in London in 1957[1]. As a consequence of the results of experiments with radioactive labels[2], the hypothesis of continuous re-utilization of large fragments of lymphocyte nucleic acids or nucleoproteins during the formation of new lymphocytes was generally accepted. Morphological support for this view has been put forward by Trowell[3], who has demonstrated that the reticular cells are differentiated into large lymphocytes in the course of phagocytosis of dead lymphocytes *in vitro*. We have been able to supplement his findings by similar observations made during the time of heightened lymphocyte dissolution *in vivo*.

I have studied the experimentally elicited disintegration of lymphocytes in spleen lymphatic nodules. The mice of *H*-strain were subjected to total-body irradiation of 500 r., 650 r., and 750 r.; rats of the Wistar strain were subjected to formaldehyde stress and normal and adrenalectomized ones to hydrocortisone ('Scheroson *F*' Shering, 4 mgm./100 gm. body-weight). X-rays were delivered at the rate of 45 r./min. (180 kV., 15 m.amp., filters 1 mm. aluminium and 0·5 mm. copper, target distance 55 cm.). The spleens were fixed in Helly–Maximow's fluid and dyed by azur II – eosin, methyl green – pyronin and by the Feulgen nuclear reaction. The specificity of ribonucleic acid staining by pyronin was controlled by means of the perchloric acid extraction technique.

In the course of dissolution of the lymphocytes, the cytoplasm of reticular cells became deeply basophilic. At the same time their nuclei and nucleoli enlarged. The strikingly close association of lymphocyte dissolution with transformation of reticular cells was seen under the following conditions.

The formaldehyde injection may elicit a stress disintegration of lymphocytes in light centres and a marked swelling of lymphoid tissue of spleen lymphatic nodules[4]. At 6 hr. after the injection, extensive

masses of large immature lymphoid cells are formed at the periphery of centres. They originate in the reticular cells occupying the corresponding part of light centres in non-stressed rats. Quite similar events are observed after the injection of hydrocortisone in adrenalectomized rats. A pronounced swelling of lymphoid tissue and disintegration of lymphocytes in the centres of the nodules occur within 3 hr. At 6 hr., groups of immature cells having large nuclei and intensive pyroninophilic cytoplasm develop. In normal rats treated by hydrocortisone, however, both the disintegration of lymphocytes and swelling of lymphoid tissue are negligible, and the immature cells fail to be differentiated.

In comparison with stress, the transformation of reticular cells is shown more clearly in a denuded reticular network of spleen lymphoid tissue of an irradiated animal. Most lymphocytes undergo disintegration and fill the spaces of the reticular stroma with numerous fragments. No phagocytosis takes place. The reticular cells adjacent to fragments show a gradual rise in cytoplasmic basophilia and the nuclei and nucleoli become enlarged as in immature cells. During this change they differ essentially from those originally building up the nodule reticulum. In the transition stages a pyronin-staining reaction may be seen around the nucleus, leaving the rest of the cytoplasm and the cytoplasmic processes almost unstained. The incidence and/or intensity of pyroninophilia of reticular cells seem to depend upon integrity of a humoral union with red pulp, in addition to an abundance of lymphocyte remnants. The pyroninophilia appears to be more intense at the periphery of the nodules than in destroyed light centres, separated from the red pulp by a dense reticular envelope. Swelling of the lymphoid tissue accompanies both the dissolution of lymphocytes and the increasing reticular pyroninophilia, as it does in the case of unspecific stress.

Eventually, the enlarged pyroninophilic reticular cells develop into more differentiated ones, mainly into immature plasma cells, but a few form large and medium-sized lymphocytes. Immature plasma cells originating in this way may occupy extensive areas of destroyed lymphoid tissue from as soon as 12 hr. after irradiation.

In mice irradiated with lethal doses of 650 r. or 750 r., and so deprived of most of their lymphocytes, the reverse 'differentiation' can be observed. At the time of disappearance of lymphocyte debris the large groups of immature cells may survive in the nodules. However, the basophilia of their cytoplasm begins to diminish until it disappears in 24–48 hr. after the irradiation. The immature cell groups undergo a dedifferentiation into a voluminous cytoplasmic syncytial mass which is not stained with pyronin. The large vesicular nuclei, in their persistence at the time of loss of pyronin stainability and of cell coupling into a syncytial feature, are proof that dedifferentiation has occurred.

These observations indicate the close relation between the dissolution of lymphocytes and the transformation of resting reticular cells into cell elements with a high power of differentiation, in accordance with the apparent signs of immaturity. The intimate local and temporal coincidence of both the dissolution of lymphocytes and the transformation of reticular cells are striking. In addition, the reverse, that is, the dedifferentiation, can also be found. The striking incidence of numerous cell fragments in proliferating centres of lymphatic nodules is stressed by DeBruyn[5] and by Ringertz and Adamson[6]. Furthermore, Dougherty and White[7] describe both dissolution of lymphocytes and an increased number of immature plasma cells as the result of adrenocortical stimulation.

These observations and results recorded in the literature suggest that dissolution of lymphocytes produces a humoral factor—probably a derivative of their nucleic acids or nucleoproteins—with the ability to maintain the cytological characteristics of immature cells on the normal level, in participating in their nucleic acid metabolism. Raising the production of such a factor in the course of heightened lymphocyte dissolution, new resting cells become transformed into immature ones; decreasing production in the case of lymphocyte deprivation, a dedifferentiation of immature cells into reticular ones occurs.

Further details of this work will be published elsewhere.

M. Hill*

Department of Histology and Embryology,
Faculty of Medicine,
University of Brno.

* Present address: Institute of Biophysics, Czechoslovak Academy of Sciences, Brno.

[1] See *Nature*, **180**, 1016 (1957).
[2] Osgood, E. E., Tivey, H., Davison, K. B., Seaman, A. J., and Li, J. G., *Cancer*, **5**, 331 (1952). Ottesen, J., *Acta Physiol. Scand.*, **32**, 75 (1954). Christensen, B. C., and Ottesen, J., *Acta Haematol.*, **13**, 289 (1955). Hamilton, L. D., *J. Clin. Invest.*, **33**, 939 (1954). *Nature*, **178**, 597 (1950).
[3] Trowell, O. A., *J. Biophys. Biochem. Cytol.*, **3**, 317 (1957).
[4] Hill, M., Pospíšil, M., and Dvořák, K., *Experientia*, **14**, 419 (1958).
[5] DeBruyn, P. P. H., *Anat. Rec.*, **101**, 373 (1948).
[6] Ringertz, N., and Adamson, C. A., *Acta Pathol. Microbiol. Scand., Supp.*, **86**, 1 (1950).
[7] Dougherty, T. F., and White, A., *J. Lab. Clin. Med.*, **32**, 584 (1947).

A Survival Curve for Mammalian Cells irradiated *in vivo*

High density single-cell suspensions of transplantable mouse leukæmia cells have been prepared from the infiltrated livers of leukæmic *CBA* mice. The density of morphologically healthy malignant cells in a suspension was determined by counting by phase-contrast microscopy, and selected serial dilutions of the counted suspension were injected intraperitoneally into groups of isologous mice. From the leukæmia mortalities in the injected groups the mean number of leukæmia cells required to convey leukæmia to half a group of injected mice (the *TD*50) was calculated by the method of Reed and Muench[1]. In six such bio-assays of suspensions from the livers of untreated leukæmic mice, *TD*50 values ranging from 0·7 to 3·0 (mean 2·0) were obtained.

The densities of morphologically intact leukæmia cells in suspensions prepared from the livers of leukæmic mice within a few minutes of the end of their exposure *in vivo* to cobalt-60 gamma whole-body radiation, delivered at a dose-rate of about 75 r./min., were found not to have been influenced by the preliminary irradiation of the mice. Bio-assays of suspensions from such irradiated mice, however, gave *TD*50 values which were raised above the mean *TD*50 (2·0 cells) obtained for suspensions from unirradiated leukæmic mice and which were a function of the radiation dose. This is to say that a proportion of the exposed leukæmic cells were found to have lost their reproductive integrity soon after irradiation without having sustained visible damage.

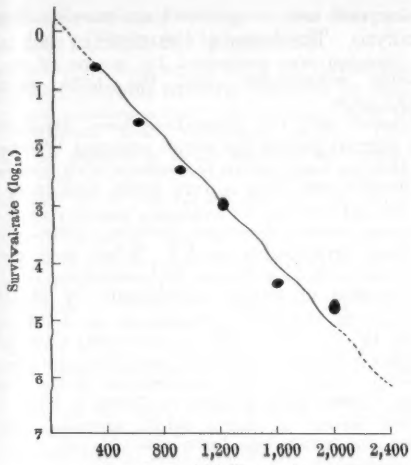


Fig. 1. Variation of log survival-rate and radiation dose for CBA leukæmia cells irradiated in vivo

The log survival-rate among the leukæmia cells from an irradiated mouse was calculated simply by subtracting the log of the *TD*50 given by the cells from the irradiated mouse from the log of the mean *TD*50 (2 cells) obtained for cells from unirradiated mice. A series of six log survival-rates was determined for cells from individual leukæmic mice which had received whole-body radiation doses between 300 r. and 2,000 r. Within the limits of experimental error, a linear relationship was demonstrated between radiation dose and log survival-rate (Fig. 1).

The curve, drawn by inspection through the points obtained by experiment, indicates a D_0 value (dose of radiation required to reduce the reproductively intact leukæmia cells to 37 per cent) of 162 r. The curve has been confirmed by a series of six further determinations of survival-rates for radiation doses within the range used initially.

In more recent experiments we have shown that the survival-rate for leukæmia cells irradiated *in vivo* in mice breathing 95 per cent oxygen was similar to that for cells from mice breathing 20 per cent oxygen during irradiation. The survival curve for cells irradiated in mice immediately after death, however, under which conditions the cells are assumed to be in an anoxic environment, showed that the anoxic cells were relatively resistant to radiation, approximately 2·3 times the dose of radiation being required for equal survival-rates.

The linear log survival curve obtained for HeLa cells irradiated *in vitro* by Puck and Marcus[3] gave a D_0 value of 96 r. for 230 kV. X-radiation. The D_0 value for leukæmia cells irradiated *in vivo*, after multiplication by the factor 0·75 to allow for the relative biological effectiveness of cobalt-60 gamma-rays compared with 230 kV. X-rays, is 122 r. The comparison indicates that the human cancer cells irradiated *in vitro* are more sensitive to radiation than the mouse leukæmia cells irradiated *in vivo*. However, incomplete studies by us have suggested that the radiosensitivity of mouse leukæmia cells irradiated *in vitro* may approach that of HeLa cells irradiated *in vitro*. The differences observed between the human and mouse cells may, therefore, prove to be due to the different conditions of the cells during irradiation rather than to the difference of species.

The work described is to be reported in detail elsewhere.

H. B. Hewitt
C. W. Wilson

Westminster Hospital School of Medicine,
Horseferry Road,
London, S.W.1.

Jan. 15.

[1] Reed, L. J., and Muench, H., *Amer. J. Hyg.*, **27**, 493 (1938).
[2] Hewitt, H. B., *Brit. J. Cancer*, **12**, 378 (1958).
[3] Puck, T. T., and Marcus, P. I., *J. Exp. Med.*, **103**, 653 (1956).

Relative Rates of Liver Storage of Pure α-, β-, γ- and δ-Tocopherols in the Growing Chick

Previous work in this laboratory on the storage of vitamin E in growing chicks has shown that only α-tocopherol was present in the liver. Since normal poultry foods often contain a high proportion of non-α-tocopherols[1,2] the ability of the chick to utilize these compounds is of importance. American workers, using the transfer of tocopherol into the egg as an index of utilization[3], found no evidence of the conversion of either γ- or δ-tocopherol to α-tocopherol, but direct measurements of liver storage were not made.

Groups of six birds of a commercial broiler strain (New Hampshire Light Sussex × White Rock) aged six weeks were placed on a purified diet low in vitamin E and depleted for a period of three weeks. Then over a period of five days they were given a series of oral doses of pure α-, β-, γ- or δ-tocopherol in ethanol–water emulsion. Two days after the final dose the birds were killed.

Blood samples were taken and whole livers were removed for tocopherol assay. Lipids were extracted using a modified form of the procedure of Swick and Baumann[4]. Saponification and removal of sterols, carotenoids and vitamin A were carried out using Brown's methods[5], and finally individual tocopherols were separated and identified by means of the two-dimensional paper chromatographic procedure of Green[3].

It was not found possible to identify any individual tocopherol in blood; but all tocopherols were found in liver with the exception of δ-tocopherol. Relative concentrations and recovery rates are given in Table 1.

The relative biological activities for α-, β-, γ- and δ-tocopherols have been quoted by Brown[5] as 100 : 30 : 20 : 1, and Ames[6] as 100 : 6 : 1 : 1 and later[7] as 100 : 33 : 1 : <1. The relative rates of liver storage quoted in Table 1 show some correlation with these values and might offer some explanation for the lower biological activities of non-α-tocopherols and especially of δ-tocopherol.

However, an additional factor in the consideration of biological activity in natural products is that of

Table 1

	Total dose 6 birds (mgm.)	Concentration found in fresh liver (μgm./gm.)	Total recovered in livers (mgm.)	Relative percentage recovery (α = 100)
α-Tocopherol	120	1·90 ± 0·19	0·289	100
β-Tocopherol	100	0·76 ± 0·17	0·099	41·2
γ-Tocopherol	120	0·34 ± 0·15	0·054	18·7
δ-Tocopherol	100	Nil	Nil	Nil

availability. Bunnell[8] has shown that the availability of α-tocopherol in lucerne to be only about 30 per cent.

I am grateful to the Directors of Spillers Limited for permission to publish this communication.

T. W. Griffiths

Spillers Limited,
Animal Nutrition Research Laboratory,
Middle Aston House,
Steeple Aston,
Oxford.
Jan. 22.

[1] Eggitt, P. W. R., and Ward, L. D., *J. Sci. Food Agric.*, **6**, 329 (1955).
[2] Green, J., *et al.*, *J. Sci. Food Agric.*, **6**, 274 (1955).
[3] Dju, M. Y., Quaife, M. L., and Harris, P. L., *Amer. J. Physiol.*, **160**, 259 (1950).
[4] Swick, R. W., and Baumann, C. A., *Anal. Chem.*, **24**, 758 (1952).
[5] Brown, F., *Biochem. J.*, **51**, 237 (1952).
[6] Ames, S. R., *Poultry Sci.*, **35**, 145 (1956).
[7] Ames, S. R., University of Maryland, Proc. Nut. Conf., March 1957.
[8] Bunnell, R. H., *Poultry Sci.*, **36**, 413 (1957).

Normal Components of Guinea Pig Serum which may cause Antigens to show an Apparent Anti-complementary Effect

Normal guinea pig serum is the most common source of complement for the complement-fixation test, and may be used fresh or, more commonly, preserved either by freeze-drying or by Richardson's method[1]. Such 'complement' is used in the presence of a great variety of antigens, and the possibility of a reaction between components of this serum other than complement and the antigen is usually either ignored or allowed for as an anti-complementary effect of the antigen acting alone.

To investigate such reactions, normal guinea pig serum was obtained by cardiac puncture from thirty animals, pooled, and preserved by Richardson's method. Following titration this serum was diluted with barbitone buffer solution, so as to contain 12·5 *MHD*50 units (minimal hæmolytic dose giving 50 per cent hæmolysis) per unit volume of 0·5 ml.

1 mgm. 'Zymosan' (Fleischmann Laboratories, Stamford, Conn., U.S.A.)[2] was added to each of two aliquots of 2 ml. of this standardized serum, and these, together with two untreated aliquots, were then incubated at 17° C. for 30 min. One of the aliquots that had been treated with 'Zymosan' was then centrifuged at 3,000 r.p.m. for 3 min. at 0° C. The supernatant fluid was decanted off and recentrifuged to ensure that all traces of the 'Zymosan' had been removed. To the final supernatant fluid 0·5 ml. barbitone buffer solution and a further 1 mgm. 'Zymosan' were added. 0·5 ml. of a 1 : 15 solution of Wassermann antigen (Burroughs Wellcome, alcoholic extract of sheep heart with cholesterol) in barbitone buffer solution was added to one of the aliquots that had not previously been treated with 'Zymosan', and also 0·5 ml. of the barbitone buffer solution to the remaining two aliquots. All four aliquots were then incubated at 37° C. for 15 min.

The amount of complement remaining after the various treatments was determined by titration. The technique used for this titration involved a serial dilution of the four solutions containing the residual complement, with a log dilution factor of 0·125. When 0·5 ml. amounts of each dilution were incubated at 37° C. for 60 min. with 2 ml. of a 0·5 per cent suspension of sheep red cells optimally sensitized with anti-sheep red cell hæmolysin (Burroughs Wellcome), several tubes were obtained within the zone of partial hæmolysis. The degree of hæmolysis in each tube of the titration was estimated by means of an EEL portable colorimeter, and the titre calculated graphically[3].

Pillemer and his co-workers have demonstrated that normal guinea pig serum contains 'Properdin'[4], and this has been shown to combine with components of preparations from a very great variety of plant and animal sources, for example, yeasts ('Zymosan'), bacteria, inulin, dextrans, gastric mucin, viruses, protozoa, trematodes, etc.[5–8]. When such preparations are used as antigens, the complexes so formed are capable of fixing complement by specifically inactivating the third component, at temperatures above 18° C. Below 18° C., however, although the complex is formed, it does not inactivate complement[4]. A factor which will fix complement in the presence of the Wassermann antigen is known to occur in the normal serum of many adult animals, although guinea pig serum has been reputed not to possess this property[9].

Table 1. The Percentage of the Initial Complement remaining following the Various Treatments

Treatment of guinea pig serum		Residual complement as percentage of initial complement (per cent)
Treated with 'Zymosan'	'Zymosan-Properdin' complex removed	46
	'Zymosan-Properdin' complex remains	23
Not treated with 'Zymosan'	Wassermann antigen added	21
	Wassermann antigen not added	63

The results presented in Table 1 show that the amount of complement fixed by the 'Properdin-Zymosan' complex, or by the Wassermann system was markedly greater than the loss of complement activity occurring during the process of incubation, or due to the presence of 'Zymosan' after most of the 'Properdin' has been removed. In the presence of an excess of antigen, this activity will vary with the amount of guinea pig serum present, independently of its complement content, unlike a true anti-complementary effect. Any allowance to be made for this effect, in addition to that made for any anti-complementary action of the antigen, must therefore be determined using the same amount of guinea pig serum as is to be used to provide the complement for the fixation tests in question.

This work was carried out while in receipt of a Colonial Veterinary Research Studentship.

M. M. H. Sewell

Department of Animal Pathology,
School of Veterinary Medicine,
University of Cambridge.
Jan. 21.

[1] Richardson, G. M., *Lancet*, ii, 696 (1941).
[2] Pillemer, L., and Ecker, E. E., *J. Biol. Chem.*, **137**, 139 (1941).
[3] Mayer, M. M., Eaton, B. B., and Heidelberger, M., *J. Immunol.*, **53**, 31 (1946).
[4] Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., and Wardlaw, A. C., *Science*, **120**, 279 (1954).
[5] Pillemer, L., Schoenberg, M. D., Blum, L., and Wurz, L., *Science*, **122**, 545 (1955).
[6] McNall, E. G., *Proc. Soc. Exp. Biol., N.Y.*, **94**, 399 (1957).
[7] Gronroos, P., *Ann. Med. Exp. Fenn.* (Helsinki), **33**, 310 (1955).
[8] Kagan, I. G., Rice Inst. Pamph., **45**, 157 (1958) (quoting Iralu, V., unpublished).
[9] Mackie, T. J., and Watson, H. F., *J. Hyg.* (Camb.), **25**, 176 (1926).

Audio-direction Finding in the Porpoise (*Phocaena phocaena*)

THE excellent anatomical work by Reysenbach de Haan[1], the important work on *Tursiops* by Scheville *et al.*[2], and numerous field observations have shown that cetaceans hear extremely well. *Tursiops* appears to possess a sonar system of remarkable quality[3] and one is inclined to believe that such a system may be of general occurrence[4], at any event among the toothed whales.

One of the striking features of hearing in the animal kingdom is the perception of the direction of sound. Experiments with man have shown that the accuracy of perception of direction is based on the difference in time the sound needs to reach both ears. In the case of pure tones and continuous tones the case is somewhat more complicated, phase differences playing a major part at lower frequencies up to about 2,000 c./s., whereas at higher frequencies differences of intensity are of special importance.

Sedee[5] has shown that in man the accuracy with which the direction of sound is determined depends, first, on the angle between this direction and the sagittal plane of the head, and, secondly, on the distance between the ears. Using an artificial head twice as large as normal, he found the threshold angle of direction to be about half its normal value.

Since sound waves travel faster in water than in air, and since the working distance between the bullæ in the porpoise is smaller than that in man, the question arises whether at lower frequencies—where differences of intensity do not play a predominant part—discrimination of direction in cetaceans may be less accurate than that in man, owing to the smaller time-differences involved. In order to get some insight into the matter, experiments were made with a porpoise.

A male porpoise was trained to come to the vicinity of the source of a sound signal by rewarding it with a fish. The underwater signal was produced by a Philips commercial tone-generator and emitted by two barium titanate transducers, one at a time. The transducers were placed at varying distances from each other and the signal was a pure tone, click-free and of short duration (± $\frac{2}{3}$ sec.). The signal was given when the animal was on a marked spot at about 18 m. from both transducers. The porpoise could only reach the vicinity of a transducer—which were separated from one another by a net—through a corridor about 12 m. long, and the animal had to choose the right opening at that distance. The enclosure in which the animal was kept had muddy slopes and eel grass on its bottom. It was therefore practically free from disturbing echoes.

It was found that the porpoise chose the right corridor if the mean angle between the transducers—one on the left side and one on the right side of the sagittal plane in front of the animal—was at least 16°. This was when a tone of 6,000 c./s. was used; at 3,500 c./s. the minimum angle is less narrow, at least 22°.

It appears from unpublished observations of Sedee, especially made on our behalf, that in man, when using the same kind of signal (but in the air), the angle in question is about 16° at 1,500 c./s. (giving the same wave-length as 6,000 c./s. in water) and about 24° at 750 c./s. The narrowest angle obtained at 1,500 c./s. was 8–10°, however, apparently half the width of that in the porpoise. As the distance between the bullæ tympani of a porpoise is about half the distance between the ears of man, it appears that, roughly speaking, a porpoise in water and a man in air have about the same degree of acuity of perception of the direction of sound when similar wave-lengths are compared.

Financial and other aid making this investigation possible was obtained from the Netherlands Organization of Pure Research (Z.W.O.), the Laboratory for Comparative Physiology at Utrecht, the Zoological Laboratory of the University of Amsterdam, the Linnaeus Fund, Amsterdam, the Verenigde Toeristen Bedrijven at Harderwijk, the Texel Museum Foundation and several Danish friends. Special thanks are due to Dr. G. A. Sedee, Utrecht, and to Mr. G. J. de Haan, Texel.

W. H. DUDOK VAN HEEL

Zoological Station, Den Helder,
Netherlands. Nov. 10.

[1] Reysenbach de Haan, F. W., *Acta Oto-Laryngolica*, Supp. 134 (Dec. 1957).
[2] Schevill, W. E., and Lawrence, B., *J. Exp. Zool.*, **124**, 1 (1953).
[3] Schevill, W. E., and Lawrence, B., *Breviora*, No. 53 (April 1956). Kellogg, W. N., Kohler, R., and Morris, H. N., *Science*, **116**, 250 (1953). Kellogg, W. N., *ibid.*, **128**, 982 (1958).
[4] Worthington, L. V., and Schevill, W. E., *Nature*, **180**, 1291 (1957).
[5] Sedee, G. A., doctorate thesis, Utrecht (1957).

PLANT PHYSIOLOGY

Effect of Light on Maturing Broad Bean Seeds

WE have recently shown[1] that the typical development of the etiolated broad bean shoot in darkness is modified by exposing the intact soaked seed to light and that the sensitivity develops at the time when the embryo takes up water. It was suggested that the embryo of the maturing seed may also be sensitive in the same way and that varying conditions of light during maturation may affect the subsequent development of the seedling.

The following experiment was carried out to test whether a light effect on the maturing embryo could be detected in seedlings grown in darkness. Plants were raised in natural light conditions, and some of the pods were enclosed in light-tight bags at an early stage when they were approximately 1 in. long. When mature the seeds were removed from the pods and dried in complete darkness. Seeds from uncovered pods were simultaneously dried in the light for the same period. All seeds were then germinated and grown in darkness; internode-lengths were measured after 13 days.

The mean length of the first internode from seeds matured in darkness (30 seedlings) turned out to be approximately 16 per cent greater than that from the controls (35 seedlings). This difference is significant at the $P = 0{\cdot}05$ level. Clearly the sensitivity develops during the maturation of the seed, and the effect survives a short drying process. The effect of light thus bears some resemblance to the vernalization effect on the embryo of winter rye in that treatment is effective in the maturing embryo[2]. The mean length of the first internode from seeds matured in the light does not differ significantly from that of controls grown in the dark from four previous experiments with commercially grown seeds (Table 1). It, in fact, lies above all the means from individual experiments.

Although the experiment was not designed to test this, the results suggest that the effect of light sur-

Table 1. Mean Length (cm.) of First Internode of Seedlings grown in Darkness for 13 Days including the Period of Soaking

	Mean length (cm.) of first internode	
	A	*B*
Seeds matured in darkness	16·4 (0·837)	—
Seeds matured in light	14·16 (0·623)	13·13 (0·235)
Seeds exposed to 2 hr. light at 24 hr. after the beginning of soaking	—	10·47 (0·251)

Column *A* from the present experiment, column *B* from previous experiments with commercially grown seeds. Figures in brackets are standard errors of the means.

vives the normal long period of inactivity in stored seeds and that the first internode of etiolated seedlings is always partially suppressed by light. It may well be that the higher figure of our controls in the present experiment reflects a reaction to the lower light-intensity in which these seeds were matured as compared with the commercially grown seeds. We hope to verify these inferences by further experiment.

This work has been supported by a grant from the Nuffield Foundation.

G. R. Lane
R. D. Butler

Department of Botany,
University of Southampton.
Jan. 28.

[1] Lane, G. R., and Butler, R. D., *Nature*, **181**, 1080 (1958).
[2] Gregory, F. G., and Purvis, O. N., *Ann. Bot.*, N.S., **2**, 237 (1938).

Differential Reaction of Saprophytic and Parasitic Soil-inhabiting Fungi to Indoleacetic Acid

Indoleacetic acid was shown to be a product of fungus metabolism, when Thimann[1] in 1935 demonstrated that this was the growth-promoting substance (for higher plants) produced by cultures of *Rhizopus suinus*. Later, attempts to reproduce in fungi the growth responses to indoleacetic acid found in higher plants were not successful[2], and interest in this field has been limited. The importance of indoleacetic acid in fungus metabolism has, however, been emphasized by several recent reports, particularly of studies of host–parasite relationships[3–6].

In the course of experiments on the effects of indoleacetic acid on two saprophytic and two plant parasitic soil-inhabiting fungi, growth of the latter was found to be much more readily inhibited than that of the former. *Ophiobolus graminis* Sacc., which causes the well-known 'take-all' disease of wheat and other Gramineae, and *Fomes annosus* Fries, which is a common cause of root and heart rot of conifers, were the two parasites studied. The saprophytes were the two soil fungi *Trichoderma viride* Pers. ex Fries, and *Trichocladium asperum* Harz.

Indoleacetic acid at various concentrations was incorporated in a synthetic liquid medium at *p*H 5·5. The medium was dispensed in 25-ml. aliquots in 200-ml. Erlenmeyer flasks. A mycelium homogenate in 0·25 *M* sodium chloride was used for inoculating the flasks and growth was measured by determining the weight of dried mycelium which developed. Typical data are given in Table 1.

Table 1. Effect of Indoleacetic Acid (IAA) on Growth of Four Fungi

IAA × 10^{-5} *M*	Dry mycelium (mgm.)			
	T. viride	*T. asperum*	*O. graminis*	*F. annosus*
0	180·9	183·7	258·4	84·6
1·0	—	180·0	224·0	46·6
10·0	—	185·5	136·5	0
50·0	169·8	203·8	0	0
100·0	188·1	183·4	0	0
L.S.D. at 1 per cent	—	—	50·4	29·1

Neither *T. viride* nor *T. asperum* was significantly inhibited by concentrations of indoleacetic acid up to 100 × 10^{-5} *M*, while *O. graminis* and *F. annosus* were significantly inhibited at concentrations of 10 and 1 × 10^{-5} *M* respectively and were completely inhibited at higher concentrations. A number of unidentified saprophytic soil fungi behaved similarly to *T. viride* and *T. asperum* in that the higher concentrations of indoleacetic acid did not significantly reduce growth.

These results may have significance for the parasitic or saprophytic development of these fungi. An extension of these findings will be published elsewhere.

E. W. B. Ward
A. W. Henry

Department of Plant Science,
University of Alberta,
Edmonton. Jan. 15.

[1] Thimann, K. V., *J. Biol. Chem.*, **109**, 279 (1935).
[2] Leonian, L. H., and Lilly, V. G., *Amer. J. Bot.*, **24**, 135 (1937).
[3] Gentile, A. C., and Klein, R. M., *Physiol. Plantarum*, **8**, 291 (1955).
[4] Pilet, P. E., *Phytopath. Z.*, **31**, 162 (1957).
[5] Shaw, M., and Hawkins, A. R., *Can. J. Bot.*, **36**, 1 (1958).
[6] Wolf, F. T., *Proc. U.S. Nat. Acad. Sci.*, **38**, 106 (1952).

Formation of Purple Pigment in *Spirogyra pratensis* Cultures

Recently, investigation of the purple vacuolar pigment of *Zygogonium ericetorum* Kütz.[1] has disclosed that it is formed by an association of ferric ions with a gallo-tannin. Similar algal pigments reported as anthocyanin-like[2] or phycoporphyrin[3] by previous investigators are probably closely related or identical to the iron-tannin of *Zygogonium*.

Most of the algal species which produce purple cell sap are found in bogs or swamps where iron is more likely to be available in rather high concentration. In addition to *Zygogonium*, several other members of the Zygnemataceae produce tannin, and tannin production by *Spirogyra* has been studied[4]. Thus it is possible that other species, not reported to form purple pigment in Nature, might form pigment under appropriate cultural conditions.

In the course of studies of *Spirogyra pratensis* Transeau, one of us (A. A.) observed that, in Waris medium containing iron sequestrene, sub-cultures of the alga in which conjugation was occurring produced purple filaments. Similar sub-cultures in soil-water remained pale green. Although extensive tests were not conducted, the distinctive responses to ammonia and to hydrochloric acid[1] indicated that the pigment which developed in the sub-cultures in Waris medium was similar to that of *Zygogonium ericetorum*.

Afterwards, vegetative filaments of *Spirogyra pratensis* were transferred into flasks of soil-water and of Waris medium. Transfers were made also into small quantitites of soil-water and of Waris medium in watch glasses enclosed within deep Petri dishes. The latter were for the purpose of encouraging conjugation so that we might compare pigment production by sexual and vegetative material. The

cultures were kept constantly at about 20° C. under cool white fluorescent lights on a 16-hr. photo-period.

The observations described here were made 35 days later. Material in flasks of soil-water was bright green while that in the flasks of Waris medium showed large patches of purple filaments among green filaments. In the watch glasses filaments in soil-water were pale yellow-green while those in the Waris medium were purple.

Filaments from the Waris medium were examined microscopically. Material in flasks of Waris medium, as well as that in watch glasses of Waris medium, showed some incipient and unsuccessfully completed conjugation. The filaments with purple pigment did not appear to be in a normal physiological state. The chromatophores were still green, but they did not have the regular spiral form typical of healthy cells. The cells were not readily plasmolysed, and the location of the pigment was in doubt. It may have been in the cell sap or perhaps adsorbed to the cell wall. There was no significant difference in the quantity of pigment formed in the material growing in Waris medium in shallow watch glasses and in flasks. Inasmuch as material in watch glasses of soil-water conjugated but did not accumulate purple pigment, it appears that the development of the pigment was not merely correlated with the occurrence of conjugation.

Although we must conclude that it has not been demonstrated that normal healthy cells of *Spirogyra pratensis* form purple pigment, the fact that the alga is capable of forming a large amount of the iron-tannin pigment in certain circumstances is of significance.

It seems now to be indicated that distinction between the algae which form purple pigment and those which do not does not depend upon their ability to synthesize a unique chromagenic substance, but rather upon their ability to tolerate an ecological niche in which iron is available in sufficient quantity to interact with the chromagen or upon their ability to accumulate the iron. There must be differences among species in their ability to produce tannins, of course; but it is obvious that, in *Spirogyra pratensis*, lack of tannin was not the limiting factor.

ANN ALLEN

Belvedere School,
Liverpool.

R. E. ALSTON

Plant Research Institute,
University of Texas,
Austin, Texas.

[1] Alston, R. E., *Amer. J. Bot.*, **45**, 688 (1958).
[2] Mainx, F., *Lotos*, **71**, 183 (1923).
[3] Lagerheim, C., *Videnskab.-Selsk. Skrifter*, I, *Mat.-nat. Kl.*, No. 5, 3 (1895).
[4] van Wisselingh, C., *Akad. van Wetenschappen Amsterdam Proc.*, Ser. C. **12**, 685 (1910).

BIOLOGY

Dissociated Nystagmus in the Rabbit

A PROMINENT feature of all forms of nystagmus is the strict co-ordination of movement of both eyes, brought about by fibre systems which cross from one side to the other. We have observed a curious phenomenon in which both eyes move synchronously but in opposite directions.

A longitudinal channel was cut in the rabbit's skull, extending caudally from the coronary suture to the region of the inferior colliculus and running along the sagittal suture. When a fine knife was guided along this channel, at a depth of 10–12 mm. from the surface of the skull, a spontaneous nystagmus, starting with a slow adduction of both eyeballs, was observed in 60 per cent of our experiments. During the rapid phase, the right eye moved to the right and the left one to the left side, that is, simultaneous contraction of both external rectus muscles occurred. The rhythm of this nystagmus was slow and irregular, but exact synchronicity was always preserved, as shown in the nystagmogram (Fig. 1).

Fig. 1. Nystagmogram of dissociated nystagmus. Upper curve, left eye; lower curve, right eye. Excursions upwards indicate movement of eyeballs to right, downwards to left. Vertical lines, time (sec.). Note irregularity and synchronicity of the dissociated nystagmus

The dissociated nystagmus lasted only for a brief period (some seconds, up to several minutes). This suggests that the acute lesion was responsible for the phenomenon. Rotation of the rabbit during the spontaneous movements, or electrical stimulation of the 'nystagmogenic centre' in the midbrain[1] produced the normal response, which superseded the dissociated nystagmus. It is thus evident that cross-connexions between left and right are still available, although the decussation, occurring in the posterior commissure[2], has been divided. This conclusion is supported by the fact that application of deeper cuts abolishes the dissociated nystagmus as well as the response to labyrinthine or central stimulation.

A full description of these experiments will be published in the *American Journal of Physiology*.

J. LACHMANN
F. BERGMANN

Department of Pharmacology,
Hadassah Medical School,
Hebrew University,
Jerusalem.
Jan. 26.

[1] Lachmann, J., Bergmann, F., and Monnier, M., *Amer. J. Physiol.*, **193**, 328 (1958).
[2] Magoun, H., and Ranson, S., *Arch. Ophthalmol.*, **13**, 862 (1935).

Strain Differentiation in a Root-infecting Fungus

IN various fields of mycology it has long been realized that, for certain purposes, the orthodox descriptive method of systematic classification is by no means adequate. Fungal strains grouped together on the bais of similar physiological reactions have been found to cut right across species of diverse taxonomic affinities, and it appears that in such disciplines as, for example, clinical medicine, agriculture, and forest pathology, the strain rather than the species is frequently of importance.

While studying the influence of certain root-infecting mycelia on the development of forest tree seedlings, unequal growth effects on the young plants were observed which strongly suggested different strains of the same fungal species as being responsible for the dissimilar performances of the seedlings. Confirmation of such strain differentiation was obtained

Table 1. Pure-Culture Reactions of Various Isolates of *Boletus scaber*

Origin of isolates of *B. scaber*	(*a*) Growth on litters	(*b*) Guaiac test	(*c*) Bavendamm test		(*d*) 'D.T.A.' medium		(*e*) Tryptophan medium	
			Growth	Reaction	Growth	Reaction	Growth	Reaction
(1) Heathland: Dorset	+	−	+	−	+	+	+	+
(2) Heathland: Surrey	+	−	+	−	+	−	(+)	−
(3) Moorland: Yorkshire	−	+	−		−		−	
(4) Woodland: Middlesex	−	+	−		−		−	
(5) Woodland: Berkshire	−	+	−		−		−	
(6) Woodland: Hertfordshire	−	+	−		−		−	
(7) Centraalbureau v. Schimmelcultures: Holland (ex Sweden)	−	+	−		−		−	

by pure culture tests of various isolates of the fungal species under observation. The species generally designated *Boletus scaber* Bull. (*Leccinum scabrum* S. F. Gray; *Krombholzia scabra* [Fr.] Karst.) was used as one of the main objects for these pure-culture tests, since two isolates of this fungus had been observed to influence the growth of young birch trees unequally[1]. Altogether, seven isolates of *B. scaber* were submitted to the tests. Two of the mycelia, (1) and (2), were derived from sporophores collected from heathland soils; another, (3), came from a sporophore on a moorland soil; (4), (5) and (6) were isolated (by M. E. Parry) from fruit-bodies of the species appearing in deciduous woods. The Centraalbureau voor Schimmelcultures in Baarn, Holland, kindly supplied isolate (7), ex Sweden.

These mycelia were tested along the following lines: (*a*) growth reactions on various broadleaf-, conifer-, and heather-litters (steam-sterilized); (*b*) gum guaiac test; (*c*) Bavendamm test (using 0·1–0·3 per cent tannic acid); (*d*) growth and colour reaction on dextrose tryptone agar with bromcresol purple ('D.T.A.' medium, supplied by Oxoid, code Nos. *G.M.* 75 and 76); and (*e*) growth and colour reaction on a tryptophan medium (using 0·1 per cent tryptophan as the sole or the additional source of nitrogen).

The results listed in Table 1 show that, on the whole, isolates (1) and (2) behave similarly. They both make good growth on a wide range of litters and give a negative reaction for phenoloxidase in the guaiac and Bavendamm tests. However, isolate (1) differs from (2) in its ability to discolour the 'D.T.A.' medium (due to a change in *p*H), also by producing a change in colour of the tryptophan medium, the change ranging from pink to cherry red, apparently caused by a metabolite derived from the tryptophan.

No interpretation of the reactions recorded in Table 1 can be put forward at this stage of the investigation. This communication is merely concerned with demonstrating the fact that the 'species' *B. scaber* does not show a uniform physiological spectrum as reflected in certain pure culture tests, which is in agreement with strain differentiation expressed in unequal effects on the growth of seedlings following inoculation of isolates (1) and (3) as reported earlier[1]. The influence of other strains of the species on the development of young trees has not been fully recorded yet.

In view of the fact that isolates (1) and (2), both from sporophores on heathland soil, react, in certain respects, similarly (Table 1) and contrast with the behaviour of the other mycelia tested, one feels tempted to suggest that differences in ecological origin might be responsible for the strain differentiation recorded. However, in order to obtain definite evidence that such a relationship exists, isolates of *B. scaber* from an additional number of localities have to be analysed and, among the ecological factors taken into account for the selection of sporophores, the tree species occurring in the neighbourhood of the fungus should be considered.

Ida Levisohn

Botany Department,
Bedford College,
London, N.W.1. Jan. 29.

[1] Levisohn, I., *New Phyt.* (in the press).

Forest Seedlings and Mycorrhiza Development on Fly Ash

The industrial ash which is a serious agricultural problem in developed countries is an even greater danger to forest stands. This is because the deposited layer is incorporated into the mineral soil in agricultural land, whereas in forests near industrial enterprises it is constantly increasing.

Table 1

	P_2O_5	K_2O	CaO	MgO
Mgm./1,000 gm. soil in 1 per cent citric acid	373·7	93·1	2,441·6	2,008·5
Percentage dry weight	0·39	0·08	1·36	0·67

Observations[1,2] on the effects of fly ash on vegetation and the discovery of toxic elements in it led us to study its effect on the forest stands. The stands were near a coal power plant in Central Bohemia using black coal. The average amounts of the main plant nutrients in the soil is given in Table 1. The *p*H of the soil was 6·36. Spectral analysis gave the following further elements: silicon, aluminium, manganese, iron, boron, tin, copper, barium, titanium, vanadium and beryllium. The presence of elements with proved toxicity in the fly ash is evident. For this reason further attention was directed to (1) the analysis of vegetation on plots covered with ash; (2) vegetation trials with seeds of forest tree species; (3) the development of mycorrhiza on seedlings from the ash layer. On plots with a heavy layer of ash acidophil vegetation (*Vaccinium myrtillus*, *Deschampsia flexuosa*, acid mosses) tend to give way to species which prefer a less acidic environment (*Fragaria vesca*, *Brachypodium sylvaticum*, *Melica nutans*) and which confirm a considerably rich layer of ash. Fly ash seems to have little effect, however, on the total number of species. Similarly, laboratory tests on Norway spruce and Scots pine showed that they developed identically on ash and on the natural substrate, namely, forest humus. An outstanding characteristic of the plots with a heavy layer of ash is the vigorous regeneration in numerous species, notably broad-leaved trees. As in the previous investigations the

ash did not appear to have an unfavourable effect, we therefore directed our attention to naturally sown seedlings, particularly with regard to the development of the mycorrhiza on their roots. Mycorrhiza, as one of the connecting links of the plant with the soil, by its susceptibility to various toxic substances[3], can give some indication of the environment in which the roots are living.

Investigations were carried out on several species; the results were as follow:

Mountain ash: endotrophic mycorrhiza with a tendency to parasitism.

Maple: without evident endotrophism; autotrophic nutrition.

Birch: typical ectotrophic mycorrhiza with strong fungus coat; partially automycotrophic nutrition.

Summer oak: typical ectotrophic mycorrhiza with fungus mantle and Hartig net on most roots. Automycotrophic nutrition prevails.

Larch (*L. decidua*): most seedlings with incomplete ectotrophic mycorrhiza; individually endotrophic mycorrhiza. Automycotrophic nutrition prevails on all seedlings.

Scots pine: on most roots only ectotrophic mycorrhiza; automycotrophic nutrition prevails.

Norway spruce: all roots with ectotrophic mycorrhiza; automycotrophic nutrition.

Hazel: well-established ectotrophic mycorrhiza.

All three stages of this work (analysis of ground vegetation in forest stands; laboratory vegetation trials; and analysis of lateral nutritive roots of natural regeneration) agree. The fly ash from black coal, although it contains apparently unfavourable elements, does not have harmful effects on forest tree species or on herbaceous vegetation. This conclusion cannot be generalized: the effect of fly ash on vegetation has to be investigated in each individual case. It is interesting that the mycorrhiza appear quite normal in an abnormal environment.

A. Sobotka
J. Materna

Forestry and Game Management Institute,
Zbraslav-Strnady,
Czechoslovakia.
Dec. 11.

[1] Rees, N. J., and Sidrak, G. H., *Nature*, **176**, 352 (1955).
[2] Holliday, R., Townsend, W. N., and Hodgson, D., *Nature*, **176**, 983 (1955).
[3] Wilde, S. A., and Persidsky, D. J., *Soil Sci. Soc. Amer. Proc.*, **20**, 107 (1956).

Analysis of Mast Cells by means of Polarization Microscopy

It has already been established that mast cells contain heparin[1], hyaluronic acid[2], histamine and 5-hydroxytryptamine[3].

Mast cells are an inhibiting factor in the clotting of blood. They are an active factor in fibrinolysis. They decompose the structure of lipids, and bind and set free histamine and 5-hydroxytryptamine. They have a cytostatic effect. They produce the ground substance of connective tissue. They exert an antitryptic effect, and enter into the metabolism of sulphur. In spite of this little is known about the structure of the protoplasm between the granules of mast cells. The problem is how to study this protoplasm, to follow its development and to investigate the connexion between the multifarious functions of

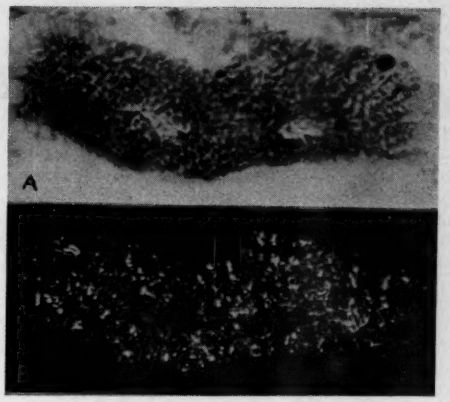


Fig. 1. Mast cell from the connective tissue of a rat. Without fixation. (*A*) Dye: 1 per mille neutral red solved in *N*/10 hydrochloric acid. (*B*) The same examined in polarized light. (× 600)

the mast cells and this protoplasm. The latter can be investigated if we change it into an anisotropic substance. We have searched for dyes which stain the granules of the mast cells well and at the same time produce anisotropy in the components of their cells, and have found that pyronin *G*, toluidine blue and neutral red (Grübler) dissolved in an acid medium (1 per thousand in *N*/10 hydrochloric acid) produce anisotropy in the intergranular protoplasm of the mast cells. The birefringence cannot be extinguished by imbibition (Fig. 1).

Preparations of membranes from the subcutaneous loose connective tissue of albino rats were used, and also rat mesentery embedded in paraffin. They were fixed by heat, chloroform – methyl alcohol or basic lead acetate. Some were stained without fixation, after drying at ordinary temperature. We did not find any difference as regards anisotropy between the mast cells in the fixed and non-fixed preparations,

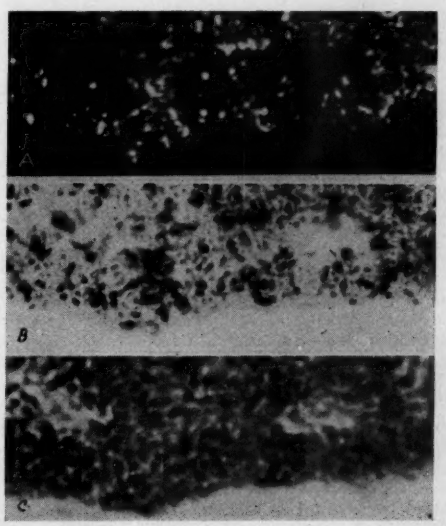


Fig. 2. Part of the cell shown in Fig. 1. (*A*) Examined in polarized light. (*B*) Contact-copy of the negative of *A*. (*C*) The same examined in normal light. (× 1,500). Note that the location of anisotropic substance in *B* is not the same as in *C*, proving that the anisotropic substance is not in the granules

or between those in the membrane preparations and those in sections.

At first it seemed that the granules of the mast cells became anisotropic; but closer analysis, however, disclosed that it was the intergranulary substance that became birefringent (Fig. 2).

The anisotropic substance is digested by ribonuclease, hyaluronidase, pepsin and trypsin. After extraction by Keilig's method[4], by which all fat-like substances are removed, birefringence is increased. This shows that the intergranulary substance is surrounded by a layer of fat-like substances which inhibit the diffusion of enzymes. But these enzymes do not digest the anisotropic substrate even after extraction by Keilig's method. Anisotropy can also be produced by using a hydrochloric acid – alcohol solution of dye. Anisotropy develops even when staining is carried out at a temperature of 80° C.

Anisotropy is prevented if saturated aqueous urea solution or 1 per cent protamine sulphate solution is used.

The dyes producing birefringence have the common property of two different poles (haptofore groups), one of which is lipophile, whereas the other is salt-producing[5]. We presume therefore that there is some complex lipoprotein material present in the intergranulary substance which possesses partly acid and partly alkyl side-chains. The side-chains of both types are oriented. The salt-producing group plays a decisive part in the development of anisotropy.

The anisotropic substance of the mast cells can be clearly followed in the connective tissue even after their disintegration.

Leslie Horváth

Department of Physiology,
School for Special Pedagogy,
Bethlen-tér 2,
Budapest, 7.

[1] Holmgren, H., *Z. mikr. anat. Forsch.*, **42**, 242 (1937).
[2] Asboe-Hansen, G., *Proc. Soc. Exp. Biol. Med.*, **79**, 651 (1952).
[3] Keller, R., *Helv. Physiol. Acta*, **15**, 371 (1957).
[4] Pears, E., "Histochemistry" (Churchill, Ltd., London, 1954).
[5] Conn, H. J., "Biological Stains" (Biotech. Pub. Geneva, N.Y., 1953).

PATHOLOGY

Oxygen Exposure and Brain Damage

In human premature infants, prolonged exposure to an oxygen concentration above 30–40 per cent in an incubator tends to produce retinal damage[1] (retrolental fibroplasia). A similar disease has been produced experimentally by oxygen exposure of newborn mice, rats, kittens and other animals, which are relatively immature at birth as compared with full-term human babies[2–4]. With the exception of pulmonary injury[5], no other pathological alterations have been reported after oxygen exposure of premature infants. As the brain, like the retina, develops considerably after birth, special attention was paid to the influence of oxygen exposure on the maturation of the brain. No characteristic brain injury has been found in children exposed to oxygen, though neurologic and mental defects are more common in children with retrolental fibroplasia than in other prematurely born children[6]. No brain damage in animals after similar treatment has hitherto been observed[3,4].

Definite alterations in the post-natal development of the cerebral cortex in mice after oxygen exposure are reported in this communication. Newborn mice were kept in 90–100 per cent oxygen continuously for 5–30 days after birth. Other mice were kept in oxygen for 5–15 days, after which they were transferred to air for 5–60 days before use. Still others, not exposed to oxygen, served as controls. More than 500 mice were used.

The relative amounts of cortical capillaries were measured on frozen sections after intracardial injection of indian ink. Continuous exposure to oxygen reduced the mass of vessels per standard area of section, the decrease amounting to 20 per cent at 15 and 20 days, in the visual as well as the motor cortex. Exposure to oxygen for 5, 10 or 15 days, followed by normal conditions, resulted in an intensified growth of the cortical vessels, in the visual and the motor areas. After 5 days in oxygen and then 15 days in air, a vascular hyperplasia was found, the relative amount of vessels exceeding that of the controls by some 10 per cent.

These alterations in cerebral vascularization are similar to the early stages in the pathogenesis of retrolental fibroplasia and of retinal damage induced by oxygen in experimental animals. In oxygen, the post-natal vascularization of the retina is suppressed. After transfer from oxygen to air, a pathological enhanced neo-vascularization of the retina takes place. Thus, similar changes occur in the cortex cerebri, though no qualitative pathological changes are seen.

In the eye, permanent retinal atrophia is often observed in adults after exposure to oxygen of newborn mice. Similarly, in cortex cerebri, the initial vascular hyperplasia after transfer from oxygen to air was followed by a slight reduction of the relative amount of cortical vessels. This vascular hypoplasia was, however, only present in the visual cortex, not in the motor cortex. It might be supposed that the vascular hypoplasia in the visual cortex depended on retinal damage and thus constituted a transneuronal alteration, due to disturbed visual function.

A second series of experiments was made in order to test this hypothesis. Growing mice were kept in complete darkness for 20 and 30 days, and the vascularity of the cortex was measured. A decrease in capillary amounts was observed, and this decrease was manifest in the visual cortex only, not in the olfactory area.

Besides studies on the vascularization of cortex cerebri, quantitative studies on the growth of the cortical cell nuclei and on the growth of internuclear material ('neuropil') were performed. Continuous exposure to oxygen for 10 days resulted in cortical intracellular œdema, indicated by nuclear swelling and an increase in the amount of neuropil per nucleus. Such cortical œdema may be of importance in the vascular hypoplasia, causing a capillary compression (as was suggested by Ashton[7] regarding similar reactions in the retina of animals exposed to oxygen). After the cortical swelling, prolonged exposure to oxygen caused a decrease in nuclear size and in neuropil growth. These alterations in the post-natal maturation of the brain cortex were observed in both the visual and the olfactory area. In contrast to the general effects on the cortex cerebri of exposure to oxygen, there was retardation of neuronal growth and of neuropil increase in the visual cortex only, not in the olfactory cortex, in animals kept in the dark for 20–30 days after birth.

Thus, exposure of growing mice to concentrated oxygen suppresses the post-natal maturation of cortex cerebri by both direct and indirect action (through retinal damage).

The present investigations will be published in full elsewhere.

LARS GYLLENSTEN

Department of Histology,
Karolinska Institutet,
Stockholm 60.
Dec. 18.

[1] Kinsey, V. E., Jacobus, J. T., and Hemphill, F. M., *A.M.A. Arch. Ophthalmol.*, **56**, 481 (1956).
[2] Gyllensten, L., and Hellström, B., *Acta Paediat.*, **43**, Supp. 100, 131 (1954).
[3] Ashton, N., Ward, B., and Serpell, G., *Brit. J. Opht almol.*, **38**, 397 (1954).
[4] Patz, A., *Amer. J. Ophthalmol.*, **38**, 291 (1954).
[5] Berfenstan, R., Edlund, T., and Zettergren, L., *Acta Paediat.*, **47**, 82 (1958).
[6] Williams, C. E., *Brit. J. Ophthalmol.*, **42**, 549 (1958).
[7] Ashton, N., Graymore, C., and Pedler, Ch., *Brit. J. Ophthalmol.*, **41**, 449 (1957).

Role of the Thymus in Murine Leukæmia

LYMPHOCYTIC neoplasms with characteristic thymic involvement occur spontaneously with high frequency in certain strains of mice[1], and in other strains following X-irradiation[2], chemical carcinogens[3] or the inoculation at birth of cell-free extracts of leukæmic tissue from high leukæmic strain mice[4]. Total thymectomy has been shown to reduce markedly the incidence of the disease, either spontaneous[1] or induced[2,3]. No report has yet appeared as to the effect of subsequent thymectomy on the occurrence of the disease in mice inoculated at birth with leukæmia extracts. Harris[5] has, however, an interesting theory which suggests that non-cellular leukæmic-inducing extracts might be capable of replacing thymic influence. Latarjet *et al.*[6] and Furth *et al.*[7] have shown that inoculation of *Ak* leukæmic cell-free extracts into newborn *Ak* mice accelerates the development of leukæmia. The effect of thymectomy on this acceleration has been investigated and reported here.

Newborn *Ak*$_i$ mice, from a colony maintained by strict sib-matings in these laboratories since 1945, were divided into three approximately equal groups, each litter being divided between the three groups. The control group had their tails cut off for identification purposes but received no further treatment apart from an intraperitoneal injection of saline. Mice in the other two groups were inoculated within 16 hr. of birth with *Ak*$_i$ leukæmic cell-free filtrates prepared according to Gross's method[4]. Extracts were made from either leukæmic thymus only, or leukæmic lymph nodes only. At 3–4 weeks of age about half the inoculated mice were thymectomized, the thymus being sucked out of the thorax through an incision in the neck. The three groups were caged side by side and maintained on ordinary laboratory diet.

The results are given in Table 1. Almost all the inoculated mice which were not thymectomized succumbed to leukæmia between 3 and 6 months of age. The majority of the untreated control mice contracted the disease but not until after the seventh month. None of the mice inoculated with either leukæmic thymus or leukæmic lymph node extracts at birth and afterwards thymectomized has yet succumbed to leukæmia. In contrast to the recent findings of Furth[8] no tumour other than leukæmia has yet been seen in any mice of the present series.

The results clearly show that in *Ak* mice inoculated at birth with cell-free extracts of leukæmic tissues, thymectomy suppressed the subsequent appearance of leukæmia. There can be little doubt as to the cell-free nature of the present extracts of leukæmic tissues for no leukæmia appeared in the thymectomized and inoculated animals, whereas it can easily be shown that thymectomy does not affect the transmission of leukæmia by cell graft. The effect of thymectomy on the incidence of leukæmia in low-leukæmic strain *C3H* mice inoculated at birth with *Ak* leukæmic cell-free extracts is under investigation and will be reported later.

Table 1. INCIDENCE OF LEUKÆMIA IN *Ak*$_i$ MICE INJECTED AT BIRTH WITH FILTRATES FROM *Ak*$_i$ LEUKÆMIC TISSUES

Treatment	Total No. of mice	Age in days at time of diagnosis					Leukæmia incidence	
		60–90	91–150	151–180	181–210	211–300	No.	Per cent
Injected at birth	29	1	18	5	2	1	27	93
Injected at birth, thymectomized	21	0	0	0	0	0	0	0
Control	34	0	0	1	3	27	31	91

I am indebted to a scholarship from the University of Queensland, Brisbane, Australia, and to Profs. A. Haddow and P. C. Koller and Dr. R. J. C. Harris for their interest. The investigations have been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Note added in proof. Since this communication was submitted for publication I have learned that similar results have been obtained in *C3Hf/Gs* mice by Drs. Levinthal, Buffett and Furth, to be published in *Proc. Soc. Exp. Biol. and Med.*

J. F. A. P. MILLER

Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
Fulham Road,
London, S.W.3.
Jan. 26.

[1] McEndy, D. P., Boon, M. C., and Furth, J., *Cancer Res.*, **4**, 337 (1944).
[2] Kaplan, H. S., *J. Nat. Cancer Inst.*, **11**, 83 (1950).
[3] Law, L. W., and Miller, J. H., *J. Nat. Cancer Inst.*, **11**, 425 (1950).
[4] Gross, L., *Cancer Res.*, **18**, 371 (1958).
[5] Harris, R. J. C., *J. Chron. Dis.*, **8**, 58 (1958).
[6] Rudali, G., Duplan, J. F., and Latarjet, R., *Bull. Assoc. franc. pour l'étude Cancer*, **44**, 440 (1957).
[7] Furth, J., Buffett, R. R., Banasiewicz-Rodriguez, M., and Upton, A. C., *Proc. Soc. Exp. Biol. and Med.*, **93**, 165 (1956).
[8] Buffett, R. F., Commerford, S. L., Furth, J., and Hunter, M. J., *Proc. Soc. Exp. Biol. and Med.*, **99**, 401 (1958).

Plaque Production with Yellow Fever and Related Arthropod-borne Viruses

WHEN a monolayer of chick fibroblasts is exposed to infection with a suitable dilution of Western Equine Encephalomyelitis virus and afterwards incubated under a layer of agar containing cell nutrients, areas of cell destruction or 'plaques' are produced; these were shown by Dulbecco[1] to result from infection by single virus particles. The plaque technique has since been applied to a number of other viruses; but

the presence of cell destruction or a cytopathic effect is normally considered to be a prerequisite for the success of the technique.

Among the arthropod-borne viruses, cytopathic changes are generally strong with group *A* viruses, such as *WEE*, but are weak or absent with group *B* viruses[2]. The demonstration by Bhatt and Work[3] that West Nile virus (which falls within the group *B*) was readily adapted to produce a cytopathic effect in tube cultures of chick embryo fibroblasts suggested that this virus might be titrated in monolayers of these cells. It was observed that early passage-levels of West Nile virus (Egypt 101 strain) which failed to produce a definite cytopathic effect in tube cultures of chick fibroblasts nevertheless yielded plaques when introduced on to chick fibroblast monolayers[4]. This observation suggested that other viruses which multiply in chick fibroblasts without producing a definite cytopathic effect might, under suitable conditions, produce plaques.

Because of its importance in the group, attention was first directed to yellow fever virus. The 17*D* strain of this virus was adapted to chick embryos by Theiler and Smith[5]. Tube cultures of chick fibroblasts support the growth of virus, but show no definite cytopathic changes. When chick fibroblast monolayers are infected with 17*D* virus under the conditions of the plaque technique, a change in the staining properties of virus-infected cells can be detected on or after the fourth day, with the development of discrete plaques 2–3 mm. in diameter. These are readily counted on the fifth or sixth day as whitish areas against a background of cells stained pink with neutral red.

In early experiments some difficulty was experienced in maintaining chick cells in a healthy state under agar for the 5 or 6 days necessary for plaque production. The technique finally adopted was to grow chick fibroblasts in a medium containing 5 per cent calf serum, 5 per cent lactalbumin hydrolysate (5 gm./100 ml.) and 90 per cent Gey's solution[6] incorporating 5 per cent sodium bicarbonate (2·25 gm./100 ml.). Overnight incubation at 37° C. of $40–50 \times 10^4$ cells in 5 ml. of medium in an atmosphere of 5 per cent carbon dioxide gave dense confluent monolayers. These were exposed to dilutions of virus in 10 per cent calf serum in Gey's solution without bicarbonate for periods of 1–4 hr. at 37° C. After removal of unadsorbed virus the plates were overlaid with a medium made by mixing equal parts of 2·0 per cent agar in distilled water and the following: Gey's solution *A* (ten times concentrated) 20 per cent, Gey's solution *B* 10 per cent, *tris* (hydroxymethyl) aminomethane 0·2 *M* *p*H 7·6 10 per cent, lactalbumin hydrolysate (5 gm./100 ml.) 10 per cent, calf serum 10 per cent, neutral red (1 : 1,000) 10 per cent, distilled water 30 per cent. Under these conditions chick cells remain healthy for seven or more days when incubated in a well-humidified incubator without added carbon dioxide. A preparation of 17*D* virus which contained 8×10^5 *LD*50 per ml. when titrated intracerebrally in mice has given titres ranging from $1·4 \times 10^6$ to $4·5 \times 10^6$ plaque-forming units per ml. according to the conditions of the test. Details of this work and of a technique for determining yellow fever neutralizing antibody will be published elsewhere[7].

The modified plaque technique was next applied to a representative selection of other group *B* arthropod-borne viruses, including the French neurotropic strain of yellow fever virus, Uganda *S*, West Nile, Wesselsbron, Japanese *B* and Louping ill viruses. West Nile, Uganda *S*, Japanese *B* and Wesselsbron viruses produced plaques which were readily visible on the second or third day and increased to 5 mm. in diameter. Zika virus resembled 17*D* in producing smaller plaques which were not visible until the fourth or fifth day. Somewhat unexpectedly, neurotropic yellow fever virus which has been maintained by serial passage in mouse brains and has never been passaged in chick tissues gave plaques which were larger and more distinct than those produced by egg-adapted 17*D* virus. Louping ill virus produced plaques which were indefinite until the seventh day. At this time they measured 2–3 mm. in diameter and were countable though less readily than with the other viruses.

There can be little doubt that the cells which make up a plaque produced by infection by *WEE* virus are in fact dead. The plaques produced by yellow fever virus have less distinct margins and frequently include a number of cells which retain the neutral red stain towards the centre of the plaque. Whether cells which lose the property of staining with neutral red are in fact dead or are in a state which is reversible and from which they normally recover in liquid media is uncertain. However, it is clear that the conditions of the plaque technique produce a change in the staining properties of virus-infected cells which represents a form of cytopathic effect which is considerably more readily detected than that normally observed by direct microscopy.

J. S. Porterfield

National Institute for Medical Research,
Mill Hill, London, N.W.7.
Jan. 17.

[1] Dulbecco, R., *Proc. U.S. Nat. Acad. Sci.*, **38**, 747 (1952).
[2] Kissling, R. E., *Proc. Soc. Exp. Biol. N.Y.*, **96**, 290 (1957).
[3] Bhatt, P. V., and Work, T. H., *Proc. Soc. Exp. Biol. N.Y.*, **96**, 213 (1957).
[4] Porterfield, J. S. (unpublished results).
[5] Theiler, M., and Smith, H. H., *J. Exp. Med.*, **65**, 767 (1937).
[6] Gey, G. O., and Gey, M. K., *Amer. J. Cancer*, **27**, 45 (1936).
[7] *Trans. Roy. Soc. Trop. Med. and Hyg.* (to be published).

BACTERIOLOGY

Bacteriophage Enrichment in a Liquid Two-Phase System with Subsequent Treatment with 'Freon' 113

The possibility of concentrating protein molecules in one of the phases in a liquid two-phase system of polymers has been investigated by Albertsson[1,2], who showed that the distribution coefficient can be related to the size of the molecules in accordance with the Bronsted[3] formula. Albertsson and Frick[4] have shown that in a dextran–methylcellulose system this relation holds for particles up to the size of phages, the upper limit at present being the size, or rather the surface area of T_2 phage. The concentration of T_2 in the bottom dextran-rich phase will be 400 times higher than in the methylcellulose-rich top phase if the two phases are allowed to separate for 48 hr. in the cold.

When phages are grown in litre quantities of substrate, it is convenient to diminish this amount to volumes more easily handled in a usual high-speed centrifuge. Essential drawbacks of the centrifugation technique (see, for example, ref. 5) are that a portion of the phages in a pellet will be broken and destroyed by the centrifugal force and that the resuspended particles will be somewhat aggregated. A more homogeneous high-titre suspension can probably be

obtained directly by the two-phase liquid enrichment technique. The necessary purification of the suspension for the removal of bacterial debris can be obtained by treatment with a commercially available fluorocarbon, 'Freon' 113.

The procedure is as follows. *Escherichia coli*, strain *B*, is grown in 2,000 ml. of a well-defined medium, Friedlein buffer[6]. The growth takes place in a container of stainless steel fitted with an outlet quite near the bottom and with a device for good aeration. The container is thermostatted at 37° C.

When the titre of *E. coli* is about 10^8, judged from absorption at 600 mμ in the Beckman *B* spectrophotometer, enough phages are added to bring the phage titre well up to 10^6. 5–6 hr. later the medium becomes fairly clear, and the phage titre is 2–5 × 10^{10}. The weight of the substrate is now adjusted back to 2,000 gm. by adding distilled water, compensating for evaporation losses. The two polymers methylcellulose ('Methocel' U.S.P. 4,000 c.p.s. from Dow Chemical Co., U.S.A.) and dextran (intrinsic viscosity 0·68 from AB Pharmacia, Sweden) are added. 601 gm. of 2 per cent methylcellulose and 131 gm. of 10 per cent dextran are weighed into the container and the contents mixed thoroughly by whirling. The container is put in the cold room (+6° C.) in an inclined position, the above-mentioned outlet being at the lowest point. After 36–48 hr., the dextran-rich bottom phase which has now formed is drawn off through a capillary stopcock. The top phase is quite clear in appearance, whereas the bottom phase is turbid. As the viscosity of the top phase is much higher, the passage of the boundary through the outlet will also show a decrease in the dropping speed from the stopcock to about one-half.

The bottom phase will have a volume of about 200 ml. containing 96–97 per cent of all phages and having a titre of 2–5 × 10^{11}, that is, about ten-fold increase in titre. The loss is due to the 3–4 per cent remaining in the upper phase. Besides this loss, there is a loss of about 10 per cent of the total compared with the total amount of phage at the time of the addition of polymers 48 hr. earlier.

The phage suspension is treated with one-third its volume of 'Freon' 113 (CCl_2F–$CClF_2$) in a Waring blendor cooled with ice[7]. The blendor is operated at high speed for 4 × 15 sec. with intervening pauses of 30 sec. After this treatment, the suspension is centrifuged at 800*g* for 10 min. The layer of denatured protein and 'Freon' will form a semi-solid pellet, and the supernatant can easily be decanted. The procedure is then repeated until after 6–7 treatments only a thin layer is formed between the 'Freon' and the dextran–water phases. In the 4–5 last treatments, the Waring blendor is operated for 4 × 25 sec.

The phages appear to withstand the 'Freon' treatment well. For six treatments the following titres were found: 1·8, 2·0, 1·6, 1·7, 1·9, 2·0 × 10^{11}. An increase after the first treatment, especially for very opaque suspensions up to 30 per cent, is often noted. It is, however, also necessary to be careful to avoid coprecipitation of phages with the denatured protein and the methylcellulose. The treatment with 'Freon' precipitates the methylcellulose, but not the dextran. (The loss in volume can be kept to 5 per cent.)

The dextran seems to have a strong protective effect, as 'Freon' treatment of phages suspended in only Friedlein buffer will result in a drop in titre of 10^3 each time. Phage suspended in full media (Difco nutrient broth) will be fully protected, but the medium will change since the treatment with 'Freon' gives a heavy precipitate in unused fresh media.

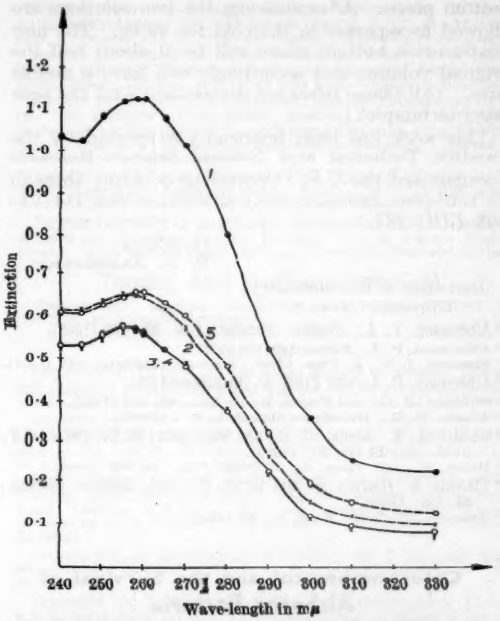


Fig. 1. Absorption of a T_4 preparation treated with 'Freon' 113. Curve 1, untreated phage suspension in dextran-rich bottom phase (titre, 3·4 × 10^{11}); curve 2, same suspension after one treatment with 'Freon' (titre, 2 × 10^{11}); curves 3 and 4, same suspension after four and six treatments with 'Freon' (titre, 2 × 10^{11}; curve 5, the values of curve 1 recalculated to the same, in this case lower, titre of curve 2. The result shows that the loss of titre corresponds to a loss in material through precipitation (probably co-precipitation). All absorption measurements on suspension, twenty times diluted with Friedlein buffer, against the same buffer

The effect of the treatment with 'Freon' on the ultra-violet spectrum of the suspension is shown in Fig. 1 and Table 1. For further purification, smaller fragments of protein can be removed by dialysis overnight against buffer. (Longer dialysis will cause losses.) If, for special purposes, it is necessary to free a suspension from all free nucleotides, this can be done by shaking with a small amount of calcium hydroxylapatite[9,10]. This operation will, however, cause some slight loss in titre. The quotients of the absorption value at 260 mμ and 280 mμ reported in Table 1 indicate a decrease in protein after treatment with 'Freon' and dialysis, and a decrease in nucleotides due to adsorption on hydroxylapatite.

To compensate for possible losses in the last operation or after sterile filtration, or to double the titre anywhere in the described procedure, 1 part of 2 per cent methylcellulose is added to 1 part of the

Table 1. QUOTIENTS OF ULTRA-VIOLET ABSORPTION AT 260 AND 280 mμ FOR A T_4 PHAGE SUSPENSION AFTER DIFFERENT TREATMENTS

	$\frac{OD\ 260}{OD\ 280}$	$\frac{OD\ 260}{OD\ 280}$ (corrected values)
Untreated suspension	1·39	1·35
1 treatment with 'Freon'	1·50	1·61
6 treatments with 'Freon'	1·53	1·63
+ dialysis	1·62	1·73
+ treatment with calcium hydroxylapatite	1·60	1·68

The values in column 2 are given after correction for background absorption calculated from ultra-violet absorption values between 330 and 370 mμ (ref. 8).

bottom phase. After shaking, the two solutions are allowed to separate in the cold for 48 hr. The new dextran-rich bottom phase will be of about half the original volume, and accordingly will have a double titre. (All phage titres are determined with the agar layer technique[6].)

This work has been financially supported by the Swedish Technical and Natural Science Research Councils and the U.S. Department of Army through its European Office, under contract number *DA*–91–508–*EUC*–287.

G. Frick
P. Å. Albertsson

Institute of Biochemistry,
Uppsala. Jan. 8.

[1] Albertsson, P. Å., *Biochim. Biophys. Acta*, **27**, 378 (1958).
[2] Albertsson, P. Å., *Nature*, **182**, 709 (1958).
[3] Bronsted, J. N., *Z. Phys. Chem.*, Bodensteinfestband, 257 (1931).
[4] Albertsson, P. Å., and Frick, G. (in preparation).
[5] Williams, R. C., and Fraser, D., *J. Bact.*, **66**, 458 (1953).
[6] Adams, M. H., *Methods in Med. Res.*, **2**, 1 (1949).
[7] Gessler, A. E., Bender, C. E., and Parkinson, M. C., *Trans. N.Y. Acad. Sci.*, **18** (2), 701 (1956).
[8] Heller, W., and Vassy, E., *J. Chem. Phys.*, **14**, 565 (1946).
[9] Tiselius, A., Hjertén, S., and Levin, Ö., *Arch. Biochem. Biophys.*, **65**, 131 (1956).
[10] Semenza, G., *Arkiv Kemi*, **11**, 89 (1957).

Chloramphenicol and the Survival of Airborne Bacteria

In recent years the large increase of staphylococcal infections in hospitals has resulted in much research into the sanitation and disinfection of hospital wards; but little work appears to have been conducted on the airborne survival of the causative agents. In the course of studies on the viability of airborne bacteria, certain observations were made which may, in part, explain the spread of these cells, especially the antibiotic-resistant strains.

Cells from each of a 24-hr. broth culture of *Staphylococcus albus*, *Serratia marcescens* and *Escherichia coli* were harvested by centrifugation, washed once and resuspended in distilled water. These suspensions were then aerosolized, by means of a Collison spray[1] into a stainless-steel rotating drum[2] of 1,100-litre capacity. Samples were collected in liquid impingers immediately after aerosolization and at subsequent hourly intervals up to 5 hr. The initial concentration in the drum was approximately 1×10^6 cells/l. of air. Loss due to physical fall-out was calculated using a modification of the radioactive tracer technique described by Harper *et al.*[3].

Viable decay-rates were calculated for the 5-hr. air suspension from the equation:

$$K = 1/t \times \ln N_0/N_t$$

where t is time of air suspension in minutes, N_0 is initial concentration of viable cells/l. of air, and N_t is concentration of cells/l. of air at time t.

The decay-rates of the antibiotic-sensitive cells were compared with (*a*) those of a resistant strain obtained from the parent strain by growth on chloramphenicol gradient plates and (*b*) with those of sensitive cells aerosolized from a suspension in a solution of 0·25 per cent chloramphenicol. In the course of study, cells were aerosolized from solutions of other bacteriostatic substances and vitamins and the results of two of these with chloramphenicol are shown in Table 1.

It can be seen that the *Staphylococci* are far more resistant to aerosol death than the two other genera, and it would appear that resistance to chloramphenicol affords considerable stability to the cells. More surprising, however, was the suggestion that a like stability could be afforded the cells by suspension in the antibiotic prior to aerosolization, and that amino-phenolic compounds were also protective. Far greater stability was afforded the cells by inositol, but considerably higher concentrations were required. In the presence of chloramphenicol, however, the quantity of inositol could be reduced by half without decreasing the stability of the cells. Thus there appeared a synergistic action between the drug and the vitamin. The observed increase in survival of cells by these compounds was found to depend on the relative humidity of the air, chloramphenicol affording greater protection to the cells at high relative humidity while inositol was more effective at low relative humidity-levels.

Table 1. Death-Rates of Some Bacteria Aerosolized from Various Media

Organism		Suspending medium			
		Water	Chloramphenicol (0·25 per cent)*	*o*-Aminophenol (0·5 per cent)*	Inositol (6·0 per cent)*
Staphylococcus albus	*S*	0·007	0·002	0·001	0·001
	R	0·002	0·003	0·002	0·002
Serratia marcescens	*S*	0·034	0·010	0·007	0·001
	R	0·012	0·008	0·008	0·002
Escherichia coli	*S*	0·029	0·013	0·006	0·001
	R	0·011	0·010	0·007	0·002

Relative humidity, 30–32 per cent; temperature, 25° C. *S*, sensitive to chloramphenicol; *R*, resistant to chloramphenicol.

* The concentrations of added compounds shown are those found to give optimal aerosol survival.

In more recent experiments other bacteriostatic drugs, such as some of the sulpha drugs, and *cyclo*-hexane substituted with hydroxyl- or amino-groups have been found to enhance bacterial aerosol survival, and the position of the substituents on the ring appears important. As a result of this work a hypothesis has been formulated that airborne cells die as a direct result of the loss of bonded water from their protein with the subsequent collapse of protein structure. It is felt (*a*) that the compounds affording increased stability to the cells are able to preserve protein structure by taking the place of water molecules during desiccation, and (*b*) that the type of bonding between protein-water and protein-compound is weak, reversible and dependent on the relative concentrations of compound and water. It is thus possible that chloramphenicol enhances bacterial aerosol survival by the same mechanism as it inhibits protein synthesis, that is, it takes the place of water molecules or blocks the water-bonding sites of the protein but does not destroy its structure, thus enzymatic action and synthesis is prevented. When, however, the drug is diluted by body fluids or water the reverse reaction occurs, water molecules displace chloramphenicol molecules and cellular metabolism is resumed.

The fact that bacteriostasis, by several organic compounds, normally used in chemotherapy and disinfection, may preserve airborne cells might, in part, explain why some measures of sanitation have failed in hospitals.

S. J. Webb

Suffield Experimental Station,
Ralston, Alberta. Jan. 9.

[1] Henderson, D. W., *J. Hyg.* (Camb.), **50**, 53 (1952).
[2] Goldberg, L. J., Watkins, H. M. S., Boerke, E. E., and Chatigny, M. A., *Amer. J. Hyg.*, **68**, 85 (1958).
[3] Harper, G. J., Hood, A. M., and Morton, J. D., *J. Hyg.* (Camb.), **56**, 364 (1958).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, April 13

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m. Discussion on "The Function and Content of an Electric Theory Course" opened by Mr. F. E. Rogers.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Lieutenant N. C. Flemming: "Last Summer's Cambridge Expedition to Explore the Submerged Ruins of an Ancient Port and City Off the Libyan Coast".

Tuesday, April 14

INSTITUTION OF BRITISH AGRICULTURAL ENGINEERS (at 6 Buckingham Gate, London, S.W.1), at 2.15 p.m.—Mr. P. H. Bailey: "The Measurement of Crop Drier Performance".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. D. L. Pratt: "The Performance of Vehicles Under Trans-Antarctic Conditions".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (joint meeting with the GRADUATE and STUDENT SECTIONS of the INSTITUTIONS OF CIVIL and MECHANICAL ENGINEERS, at Savoy Place, London, W.C.2), at 6.30 p.m.—The Rt. Hon. the Viscount Chandos, P.C., D.S.O., M.C.: "Finance and the Engineer".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. W. Feitknecht (University of Berne): "Studies of the Influence of Chemical Factors on the Corrosion of Metals" (Spring Lecture).

INSTITUTE OF METALS (in the Lecture Theatre of the Royal Institution, Albemarle Street, London, W.1), at 7 p.m. Prof. F. Hoyle, F.R.S.: "The Scientific Uses of Space Exploration".

Tuesday, April 14—Wednesday, April 15

LABORATORY ANIMALS CENTRE (at the Royal Veterinary College, Royal College Street, London, N.W.1)—Twelfth L.A.C. Congress.

Wednesday, April 15

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Prof. George C. Varley: "The Biological Control of Agricultural Pests" (Fernhurst Lecture).

PHYSICAL SOCIETY, COLOUR GROUP (at the Victoria and Albert Museum, South Kensington, London, S.W.7), at 3.15 p.m.—Annual General Meeting. Mr. J. W. Perry: "Psychophysical Measurement" (Chairman's Address).

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. P. A. Stone: "The Economics of Housing and Urban Development".

UNIVERSITY COLLEGE (in the Chemistry Theatre, Gower Street, London, W.C.1), at 6 p.m.—Colonel H. T. Jessop: "Photoelasticity at University College London: Jubilee Commemoration of the Work of E. G. Coker and L. N. G. Filon, 1909–1937"; with an Exhibition of Work in Photoelasticity at University College London, in the Department of Physics (also open April 16 and 17).*

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Dr. J. B. Stenlake: "Progress in the Chemistry of Natural Products".

Thursday, April 16

LABORATORY ANIMALS CENTRE (at the Royal Veterinary College, Royal College Street, London, N.W.1), at 9.30 a.m.—Symposium on "Quality in Laboratory Animals".

ROYAL SOCIETY (at Burlington House, Piccadilly London, W.1), at 4.30 p.m.—Prof. E. L. Hirst, F.R.S.: "Molecular Structure in the Polysaccharide Group" (Bakerian Lecture).

INSTITUTION OF MINING AND METALLURGY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. F. Mendelsohn: "Structure of the Road Antelope Deposit".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. Kenneth Sporne: "Correlation of Biological Characters"; Prof. H. Grüneberg, F.R.S.: "On Correlation of Characters in the Mouse".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. D. A. Edwards: "Commutative Algebras of Operators".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. O. Bishop and Dr. G. S. Brosan: "An Electromagnetic, Variable-Ratio, Torque Convertor".

ROYAL ANTHROPOLOGICAL INSTITUTE (at 21 Bedford Square, London, W.C.1), at 5.30 p.m.—Dr. G. Ainsworth Harrison: "Environmental Determination of Mammalian Morphology".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. R. I. Hughes: "Adhesion in Bitumen Macadam".

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, Portland Place, London, W.1), at 6 p.m.—Annual General Meeting, followed by Mr. A. P. Roberts: "Self-Optimising Control System for a Certain Class of Randomly Varying Inputs".

BRITISH COMPUTER SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Prof. L. Hogben, F.R.S.: "The Mechanical Translation of Languages".

BRITISH INSTITUTION OF RADIO ENGINEERS, RADIO GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Inaugural Meeting.

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion on "Problems of Recording and Communicating Technical Information in a Commercial Research Organization", opened by Miss Pamela D. Waterhouse.

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at the Royal Institution, Albemarle Street, London, W.1), at 6.30 p.m.—Prof. A. Stoll: "The Cardiac Digitalis Glycosides".

BRITISH INSTITUTE OF RADIOLOGY, incorporated with the RONTGEN SOCIETY (at 32 Welbeck Street, London, W.1), at 8 p.m.—Prof. G. Stead: "1895 and All That" (Silvanus Thompson Memorial Lecture).

Thursday, April 16—Thursday, April 30

ENGINEERING, MARINE, WELDING AND NUCLEAR ENERGY EXHIBITION (at Olympia, London).*

Friday, April 17

INSTITUTE OF PHYSICS (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 9.45 a.m.—Symposium on "Current Developments in the Production of High Vacua".

PLASTICS INSTITUTE (joint meeting with the LONDON SECTION of the INSTITUTION OF THE RUBBER INDUSTRY, in the William Beveridge Hall of the London University, Malet Street, London, W.C.1), at 10 a.m.—Conference on "Adhesion".

ASSOCIATION OF APPLIED BIOLOGISTS (in the Metallurgy Lecture Theatre, Royal School of Mines, Imperial College, Prince Consort Road, London, S.W.7), at 2.15 p.m.—Annual General Meeting, followed by Dr. I. Thomas: "Control and Conservation" (Presidential Address).

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4 p.m.—Symposium on "Biomechanics".

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Colonel C. Powell: "The Dectra Trials".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR LECTURER IN ORGANIC CHEMISTRY; a LECTURER IN CHEMICAL TECHNOLOGY; LECTURERS (2) IN MATHEMATICS; an ASSISTANT (Grade B) IN MATHEMATICS; LECTURERS (2) IN PHYSICS; and an ASSISTANT (Grade B) IN PHYSICS—The Registrar, College of Technology, Ashley Down, Bristol 7 (April 16).

ASSISTANT, Grade A (with a degree or equivalent qualifications), IN THE DEPARTMENT OF PURE AND APPLIED SCIENCE, Northampton College of Technology, to teach chemistry and physics—The Chief Education Officer, Borough Education Office, "Springfield", Cliftonville, Northampton (April 18).

LECTURER, Grade II (a) (with a good honours degree in physics and teaching and/or industrial experience) IN PHYSICS at Dundee Technical College—The Clerk and Treasurer, Institute of Art and Technology, 40 Bell Street, Dundee (April 18).

LECTURER IN THE DEPARTMENT OF METALLURGY, to teach and carry out research in Physical Metallurgy—The Registrar, The University, Liverpool (April 18).

ASSAY MASTER—Sir Douglas S. Branson, Law Clerk to the Guardians, Assay Office, 6 Paradise Square, Sheffield 1 (April 20).

LECTURER or ASSISTANT LECTURER (with a good honours degree in chemistry and special qualifications in physical chemistry) IN CHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 20).

ASSISTANT LECTURERS (2) IN PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (April 22).

LECTURER (with a good honours degree and some experience in research and in industry) IN CHEMICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 22).

ASSISTANT LECTURER IN PURE OR APPLIED MATHEMATICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (April 25).

CHAIR OF SOCIOLOGY—The Registrar, The University, Leicester (April 25).

LECTURER or ASSISTANT LECTURER IN PHYSICS—The Registrar, Manchester College of Science and Technology, Manchester 1 (April 25).

SENIOR LECTURER IN POLITICAL AND SOCIAL GEOGRAPHY—The Registrar, The University, Leicester (April 25).

ASSISTANT LECTURER (with a degree in botany with some zoology) IN BIOLOGY—The Registrar, University College of North Staffordshire, Keele, Staffs (April 30).

ASSISTANT LECTURERS IN PURE MATHEMATICS, ELECTRONIC ENGINEERING, CHEMISTRY (INORGANIC and PHYSICAL), AGRICULTURE (ANIMAL GENETICS), MARINE BIOLOGY and PSYCHOLOGY—The Secretary and Registrar, University College of North Wales, Bangor (April 30).

LECTURER (honours graduate in science, with qualifications in two of the following: botany, chemistry or geography) IN EDUCATION—The Secretary and Registrar, University College of North Wales, Bangor (April 30).

SENIOR LECTURER IN THE DEPARTMENT OF PHYSIOLOGY, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, April 30).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Hull (May 1).

LECTURER (graduate, preferably with honours or higher degree in chemistry) IN CHEMISTRY in the Department of Science and Metallurgy—The Principal, North Lindsey Technical College, Kingsway, Scunthorpe, Lincs (May 4).

LECTURER IN NUCLEAR ENGINEERING—The Registrar, Queen Mary College, Mile End Road, London, E.1 (May 6).

LECTURER or ASSISTANT LECTURER IN INORGANIC AND STRUCTURAL CHEMISTRY—The Registrar, The University, Leeds 2 (May 6).

CHAIR OF APPLIED MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 8).

SENIOR LECTURER IN MEDICINE at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, May 9).

LECTURER (with British university qualifications, or equivalent, and preferably experience in teaching geography in a university department of geography and geology) IN GEOGRAPHY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, May 15).

SENIOR LECTURER (with an appropriate higher qualification and experience in teaching and in research methods) IN MEDICINE at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, May 15).

CHAIR OF PHYSIOLOGY in the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, May 30).

COMPUTING ASSISTANT (female, with at least A-level mathematics or equivalent, with hand computing experience), for work in connexion with rocket and satellite studies of the upper atmosphere—The Secretary, University College, Gower Street, London, W.C.1.

EXPERIMENTAL PHYSICISTS (with at least a good second-class honours degree, and preferably postgraduate experience in some aspect of nuclear physics), for (*a*) to collaborate with physicists designing a 7 GeV. Proton Synchrotron, to take responsibility for aspects of selection, analysis and transport of high energy beams and eventually to participate in high energy nuclear research; and (*b*) to work with nuclear physicists using a 50-MeV. Proton Linear Accelerator shortly to be commissioned and to collaborate with university physicists using this machine—The Secretary, National Institute for Research in Nuclear Science, Rutherford High Energy Laboratory, Harwell, Didcot, Berkshire, quoting N.1/1/1388/34.

FISHERIES RESEARCH OFFICER (with a good honours degree in natural science (zoology) and training or experience in fish ecology) in Nyasaland, for research into the biology and ecology of Lake Nyasa fish—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.152/4/01.

SCIENCE MASTER TO TEACH PHYSICS to A and CHEMISTRY to O level—The Headmaster, Cheltenham College.

SCIENTIFIC ASSISTANT (ABSTRACTOR) (with a degree in agriculture or natural science, and a knowledge of languages)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

SENIOR LECTURERS and LECTURERS IN MATHEMATICS AND STATISTICS—The Registrar, Bradford Institute of Technology, Bradford 7.

SENIOR PLANT PATHOLOGIST (with a good honours degree in botany, considerable experience in plant pathology, and administrative ability) in Northern Nigeria, for general plant pathology investigations—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/408/039.

SENIOR TECHNICIAN (with experience in experimental physiology) IN THE PHYSIOLOGY DEPARTMENT—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1.

ZOOLOGIST (with at least an honours degree in zoology, biology or entomology of a recognized university, plus at least two years approved postgraduate experience) at the Institute of Medical Research, Kuala Lumpur, Federation of Malaya, for duties which involve research on the ecology of arthropod vectors and on animals as disease reservoirs, with special reference to arthropod-borne virus diseases—The Recruitment and Personnel Division, Government of the Federation of Malaya, Malaya House, 57 Trafalgar Square, London, W.C.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Building Research Station Digest No. 118 (January, 1959): Reducing Laboratory Vibrations. Spray Taps. Pp. 4. (London: H.M. Stationery Office, 1959.) 4*d*. [112

Economic Proceedings of the Royal Dublin Society. Vol. 4, No. 6 (October 8, 1958): Population Studies on Cereal Root Eelworm *Heterodera major* (O. Schmidt, 1930). By J. J. Duggan. Pp. 103–118 + plate 17. (Dublin: Royal Dublin Society, 1958.) 4*s*. [112

Bardsey Bird and Field Observatory. Report for 1957. Pp. 44. Egluryrafach, Machynlleth, Mont.: W. M. Condry, Hon. Secretary, (1959.) [112

Proceedings of the Royal Irish Academy. Vol. 59, Section A, No. 3: Expansions for the Logarithmic-Integral and Cosine-Integral Functions. By A. S. Meligy. Pp. 25–28. 6*d*. Vol. 59, Section A, No. 4: Structure Theory. By D. J. Newman. Pp. 29–47. 2*s*. Vol. 59, Section B, No. 12: The Littoral Harpacticids of the Dalkey (Co. Dublin) Area with Descriptions of Six New Species. By Katharine M. Roe. Pp. 221–255. 5*s*. Vol. 59, Section B, No. 13: Studies of Flowering-Plant Growth and Organo-Genesis—III: Leaf Shape Changes Associated with Flowering and Sex Differentiation in *Cannabis sativa*. By J. Heslop-Harrison and Y. Heslop-Harrison. Pp. 257–283. 4*s*. Vol. 59, Section B, No. 14: The Carboniferous Knoll-Reefs of East-Central Ireland. By W. B. Nevill. Pp. 285–303 + plate 5. 2*s*. 6*d*. Vol. 59, Section B, No. 15: The Chemical Composition of some Irish Peats. By T. Walsh and T. A. Barry. Pp. 305–328. 3*s*. 6*d*. Vol. 59, Section B, No. 16: Pollen Analytical Investigations of the Mire at Fallahogy TD., Co. Derry. By A. G. Smith. Pp. 329–343 + plates 6–9. 4*s*. Vol. 59, Section B, No. 17: Antitubercular Substances—XV: Some Derivatives of Tetrahydrofuran. By Vincent C. Barry and Joan E. McCormick. Pp. 345–351. 1*s*. (Dublin: Hodges, Figgis and Co., Ltd., 1958.) [113

Other Countries

Chicago Natural History Museum. Fieldiana: Zoology. Vol. 39, No. 24 (December 18, 1958): Three New Skinks Related to *Sphenomorphus variegatus* (Peters). By Robert F. Inger. Pp. 257–268. (Chicago: Chicago Natural History Museum, 1958.) 30 cents. [102

United States Department of Agriculture. Agriculture Handbook No. 149: Growing Lettuce in Greenhouses. By Ross C. Thompson, S. P. Doolittle and T. J. Henneberry. Pp. 22. 15 cents. Production Research Report No. 23: Pitting for Range Improvement in the Great Plains and the Southwest Desert Regions. By Oscar K. Barnes, Darwin Anderson and Arnold Heerwagen. Pp. 17. 10 cents. (Washington, D.C.: Government Printing Office, 1958.) [102

Florida State Museum. Report of the Director for 1957–58. Pp. 20. (Gainesville, Fla.: Florida State Museum, The University, 1959.) [102

The United States National Museum: Smithsonian Institution. Annual Report for the year ended June 30, 1958. Pp. iv + 150. (Washington, D.C.: Government Printing Office, 1958.) [102

International Atomic Energy Agency. Safety Series No. 1: Safe Handling of Radio-isotopes. Pp. 100. (Wien: International Atomic Energy Agency, Kärtner Ring 11, 1958.) 6*s*.; 1 dollar; 350 French francs. [102

International Conciliation, No. 521 (January, 1959): The International Geophysical Year. By Walter Sullivan. Pp. ii + 259–336. (New York: Carnegie Endowment for International Peace, 1959. Copies obtainable from Columbia University Press, 2960 Broadway.) 25 cents. [102

Kungl. Skogshögskolans Skrifter. (Bulletin of the Royal School of Forestry, Stockholm.) Nr. 31: Sønderdelingsprocesser i sur Humus. Av E. Oksbjerg. (Formation of Carbondioxide in Raw Humus Samples.) Pp. 30. (Stockholm: Kungl. Skogshögskolan, 1958.) [102

Durban Museum and Art Gallery. Annual Report for the Municipal Year 1957–1958. Pp. 19. Durban Museum Novitates. Vol. 5, Part 11, (15th December, 1958): A New Race of Tomb Bat *Taphozous perforatus*, E. Geoffroy, 1818, from Northern Nigeria, with some Observations on Its Breeding Biology. By David L. Harrison. Pp. 143–149. 2*s*. 6*d*. (Durban: Durban Museum and Art Gallery, 1958.) [102

Illinois Natural History Survey Bulletin, Vol. 27, Article 2 (December, 1958): A Century of Biological Research. By Harlow B. Mills, George C. Decker, Herbert H. Rose, J. Cedric Carter, Bessie B. East, George W. Bennett, Thomas G. Scott, James S. Ayars and Ruth R. Warrick. Pp. vi + 85–234. (Urbana, Ill.: Illinois Natural History Survey, Natural Resources Bldg., 1958.) [102

Report of the South African Museum for the year ended 31st March, 1958. Pp. 19. (Cape Town: South African Museum, 1958.) [102

Smithsonian Miscellaneous Collections. Vol. 138, No. 4: Birds of the Pleistocene in North America. By Alexander Wetmore. Pp. ii + 24. (Washington, D.C.: Smithsonian Institution, 1959.) [102

Proceedings of the United States National Museum. Vol. 108, No. 3403: Rhizocephala of the Family Peltogastridae Parasitic on West Indian Species of Galatheidae. By Edward G. Reinhard. Pp. 295–307 + plate 1. Vol. 108, No. 3405: Three New Serranid Fishes, Genus *Pikea*, from the Western Atlantic. By Leonard P. Schultz. Pp. 321–329. Vol. 108, No. 3406: The Status of the Lizard *Cnemidophorus perplexus* Baird and Girard (Teiidae). By T. Paul Maslin, Richard G. Beudleman and Charles H. Lowe, Jr. Pp. 331–345. (Washington, D.C.: Government Printing Office, 1958.) [102

Institut Royal des Sciences Naturelles de Belgique. Mémoires. No. 139: Étude Comparative de la Croissance de la Mandibule chez l'Homme et les Singes Anthropoïdes. Position des Mandibules d'Hommes Fossiles par Rapport a ces Formes Actuelles. Par Nicole Petit-Maire-Heintz. Pp. 64. No. 140: Écologie d'Une Prairie Marécageuse (Chênée—Sauheid, Province de Liège.) Par Serge Jacquemart et Eugène Leloup. Pp. 69 + 3 planches. No. 141: Contribution a l'Étude du Dinantien de la Belgique. Par F. Demanet. Pp. 152. No. 142: Les Foraminifères Mio-Pliocènes et Quaternaires du Kruisschans. Par Johannes H. van Voorthuysen. Supplément sur Quelques Rotalidés par Jan. Hofker. Pp. 63 (10 planches). No. 143: Foraminifera of the Oligocene of Belgium. By D. A. J. Batjes. Pp. 188 + 13 plates. Deuxième Série. Fasc. 56: Revision du Genre Gasteruption (Hymenoptera, Evanoidea, Gasteruptionidae). Espèces Australiennes. Par Prof. Jean Pasteels. Pp. 125. Fasc. 57: Revision der Gattung Microstigma Rambur (Ordnung Odonata, Zygoptera). Von Dr. Erich Schmidt. Pp. 44 + 8 plates. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1958.) [102

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office